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PRELIMINARY SEROLOGICAL OBSERVATIONS ON DOGS ON AN ATHEROGENIC REGIMEN

SAUL P. BAKER, ERIC OGDEN, AND JACKSON W. RIDDLE*

The presence of cholesterol in human atheromata has led to the study of cholesterol intake and serum cholesterol concentration in attempts to elucidate the pathogenesis of atherosclerosis in man and experimental animals. Correlations between diet or serum cholesterol concentration and incidence of atherosclerosis in man have been indecisive. The rabbit, a herbivore, readily lays down cholesterol atheromata when its cholesterol intake is raised. The chicken, with much more catholic dietary habits, regularly shows spontaneous atheromatosis, but an artificial increase of its cholesterol intake will increase the incidence of these lesions and change their distribution. Steiner and Kendall have produced atherosclerosis in dogs by raising their cholesterol intake while on thiouracil. Presumably, the thiouracil contributes to this effect by depressing thyroid function.

On the basis of a study of the sera of man and experimental animals, Gofman has indicated that mainly that fraction of the cholesterol, whether autogenous or ingested, which is present in the serum in the form of lipoproteins of a specific flotation range (S_f 10–20) is associated with the production of atherosclerosis. If this concept is valid, and since the susceptibility of individuals and of species to atherosclerosis varies, it is feasible to consider the possibility that these variations are due to changes in the availability of the particular protein which enters into this lipoprotein combination.

The techniques of immunology have made the greatest contribution to our ability to recognize specific individual variations in proteins. For this reason, an attempt is being made to explore the possibility that the chemical entities in Gofman's S_f 10–20 lipoprotein fraction may be studied immuno-

*From the Departments of Physiology and Bacteriology, College of Medicine, The Ohio State University, Columbus, Ohio. Received for publication May 10, 1951.

logically and correlated with atherosclerosis. This report indicates from a preliminary survey that such an approach is worth further study.

Procedure. Three male mongrel dogs (A, B, and C) weighing from 6 to 8 kg. were placed on dietary regimens of cholesterol (Eastman—10 g. daily) and thiouracil (Lilly—1.2 g. daily); cottonseed oil (30 ml. daily) and thiouracil (1.2 g. daily); respectively, in addition to their stock diet of Purina dog chow and horsemeat. These diets were designed to produce varying degrees of atherosclerosis. Three male mongrel dogs (D, E, and F) on stock diets of Purina dog chow and horsemeat were used as controls.

After the experimental dogs had been on these diets for 7 months, their sera were fractionated in the following manner, based on Gofman's ultracentrifugal technique for isolation of lipoprotein molecules.

Three ml. of sodium chloride solution of specific gravity 1.063 were added to 2 ml. of dog serum. This mixture was then centrifuged for 30 minutes at a speed of 15,000 r.p.m. in an International Refrigerated Centrifuge at a temperature of 35° F. This serum fraction was individually prepared from each of the six dog sera (A, B, C, D, E, and F). After centrifugation, 2 ml. of the supernatant fluid were drawn off by capillary pipette, diluted to 5 ml. with distilled water, and

TABLE I

Comparison of Precipitin Reactions Obtained with Immune and Control Rabbit Sera Against Experimental and Control Dog Sera and Against Human Sera Rabbit Serum

Serum Dog	[a] Dil.	Ppt.	[b] Dil.	Ppt.	[c] Dil.	Ppt.	[d] Dil.	Ppt.	[e] Dil.	Ppt.	[f] Dil.	Ppt.
A	**1:10000**	3+	1:10000	3+	1:10000	1+	1:100	1+	1:100	1+	1:10	1+
B	1:10000	2+	1:10000	2+	1:10000	1+	1:100	1+	1:10	1+	1:10	±
C	1:5000	3+	1:5000	3+	1:5000	1+	1:100	1+	1:10	±	1:10	±
D	1:5000	1+	1:1000	2+	1:1000	1+	1:10	1+	1:10	±	1:10	±
E	1:5000	1+	1:1000	1+	1:1000	1+	1:10	1+	1:10	±	1:10	±
F	1:5000	1+	1:1000	1+	1:1000	1+	1:10	±	1:10	1+	1:10	±
Human												
G	1:1000	2+	1:1000	1+	1:100	1+	1:10	—	1:10	—	1:10	—
H	1:1000	2+	1:1000	1+	1:100	1+	1:10	—	1:10	—	1:10	—
I	1:1000	2+					1:10	—	1:10	—	1:10	—
J	1:1000	2+					1:10	—	1:10	—	1:10	—

See text for classification of sera.

injected intravenously into six young adult rabbits (a, b, c, d, e, and f—corresponding to the dogs with the same letters) at seven day intervals for a total of three injections. Seven and twelve days after the last injection, rabbits a, b, and c and rabbits d, e, and f, respectively, were bled aseptically from the heart. The sera obtained from these six rabbits (a, b, c, d, e, and f) were used in precipitin tests with the untreated sera from the 6 dogs (A, B, C, D, E, and F), and in later precipitin tests with sera from 3 normal men in the 25–35 year-old age group (G, H, and I) and from a 57 year-old man proven to have had a recent (2 weeks) myocardial infarct (J).

Results. Results obtained from these preliminary tests (Table I) are as follows:

(1) The serum fractions from dogs, A, B, and C contained substances which were antigenic in rabbits a, b, and c, respectively.

(2) The concentration of these antigens in both treated and untreated sera from dogs A and B was clearly greater than in the control dogs (D, E, and F). Dog C, on thiouracil, but with no added lipid, showed an intermediate concentration of antigen.

(3) The serum fractions from control dogs D, E, and F did not significantly immunize rabbits d, e, and f against the sera of either the test dogs A, B, and C or the control dogs D, E, and F.

(4) The immune rabbit sera (a, b, and c) reacted with all of the human sera tested in a range comparable to that obtained with the control dog sera (D, E, and F), thus demonstrating a concentration of these antigens in human sera as well as in dog sera. This excludes the possibility that the phenomenon reported is characteristic of the species rather than of the dietary regimen.

(5) The sera from the control rabbits (d, e, and f) produced no reaction with any of the human sera tested.

Discussion. The evidence presented here suggests the possibility of quantitative immunological studies of the serum alterations associated with atherosclerosis. Further studies are now in progress to develop other serological techniques in order to refine the methods of detection and to quantitate

Our own interest was aroused by the increased number of isolations of this organism from patients in the John Sealy Hospital of Galveston during the past year. The present study was undertaken in the hope that a satisfactory drug or drugs might be uncovered for the treatment of moniliasis. Existing methods of treatment have been disappointing. For local infections they consist largely of alkaline washes, painting with gentian violet, potassium iodide, potassium permanganate soaks, ammoniated mercury ointment, sodium propionate ointment, or vaccine desensitization. Systemic infections are treated with potassium iodide, and for patients showing sensitivity to the organism a desensitization with graduated doses of a vaccine may also be used.

We have screened forty drug compounds for monilia inhibitory effect *in vitro*, and have tested the most promising of these drugs with *Candida* inoculated rabbits. Through the courtesy of Dr. Levin, we also obtained clinical evidences on the efficacy of this drug.

In Vitro Methods

Accurately weighed quantities of various compounds were dissolved in water or alcohol and sterilized, either by autoclaving at ten pounds pressure for ten minutes, or in more instances, by Seitz filtration. Appropriate dilutions were then made, preferably in water, and added to the measured volumes of sterile broth. The medium was then inoculated and incubated for 48 hours at 35° C. In cases where alcohol had to be used as a solvent, alcohol controls were included.

Bacto tryptose broth with 0.5 per cent sodium chloride was used as the test medium throughout the investigation except as otherwise specified. Measured amounts of broth were dispensed into test tubes and sterilized at fifteen pounds for fifteen minutes. Broth-drug dilutions were inoculated with one drop of a twenty-four hour broth culture of the test organism from a one ml. pipette, and the resultant growth was read by comparison with a control tube containing no drug.

All drugs were tested against at least four strains of *C. albicans*. Three of the cultures used in this investigation were obtained from Dr. N. F. Conant, Duke University, and the

others were strains isolated from patients in the John Sealy Hospital.

In Vitro Results

Table I gives the results of seven antibiotics against *C. albicans*. Of these antibiotics, only Thiolutin (Pfizer) showed promise. It gave complete inhibition at 10 micrograms per ml. In other instances the inhibiting concentrations were far in excess of the established therapeutic levels. It had been hoped that the inhibitory concentrations would be low enough to try a combination of one or more antibiotics.

TABLE I

In Vitro Studies on Seven Antibiotics on the Growth of C. Albicans

Antibiotic	Concentration giving complete inhibition of growth (micrograms per ml.)
Thiolutin (Pfizer)*	10
Neomycin sulfate	> 1000
Aureomycin hydrochloride	> 500
Streptomycin sulfate	> 1000
Penicillin G Potassium	> 1000
Chloromycetin	> 1000
Terramycin*	> 500

*Kindly supplied by Dr. Elliott R. Weyer, Charles Pfizer and Co.

In Table II are the results of several miscellaneous compounds tested *in vitro*. Potassium iodide is accorded some efficacy against fungus infections, but even 1 mg. per ml. failed

TABLE II

In Vitro Studies of Miscellaneous Drugs on the Growth of C. Albicans

Drug	Highest concentration tested	Growth
Sodium sulfadiazine	10 mg. %	4+
Potassium iodide	1000 μg/ml.	4+
Para-amino-benozoic acid	1000 μg/ml.	4+
Para-amino-salicylic acid	5 mg/ml.	4+
Promin (SN 166) (p,p'-diaminodiphenyl-sulfone-N,N'-di-(glucose sodium sulfonate)	1000 μg/ml.	4+
Paludrine (SN 12837) (1-(p-chlorophenyl)-4-isopropylbiguanide)	1000 μg/ml.	4+
Metachloridine (SN 11437) (N'-(5-chloro-2-pyrimidyl) metanilamide)	2000 μg/ml.	4+

4+ = growth equal to that of control tube.

to inhibit growth of *C. albicans*. Para-amino-salicylic acid was completely ineffective in ranges up to 5 mg. per ml., para-amino-benzoic acid up to 1,000 micrograms per ml., and such miscellaneous antimalarial drugs as Promin, Paludrine and metachloridine in ranges tested. Sodium sulfadiazine was found completely ineffective at concentrations of the established therapeutic levels.

Table III shows the results of acridine and various acridine derivatives in their efficacy to inhibit the growth of *C. albicans*. Of these drugs, Acriflavine and Rivanol showed greatest inhibition; Acriflavine giving complete inhibition at 10 micrograms per ml. and Rivanol at 10–20 micrograms per ml. Gomez-Vega (1935) found Acriflavine effective against *C. albicans* in a 1:5000 dilution. Acridine was not tested in higher concentrations than 20 micrograms per ml. because of its insolubility; neither were Acranil and Neo-Acranil tested in higher concentrations than this.

TABLE III

In Vitro Studies of Acridine and Various Acridine Derivatives on the Growth of C. Albicans

Drug	Concentration giving complete inhibition of growth (micrograms per ml.)
Acridine	> 20
Acriflavine (3,6-diamino-acridine-10-methochloride hydrochloride)	10
Rivanol lactate (2-ethoxy-6,9-diamino-acridine-lactate) 2 strains	10
2 strains	20
Atabrine dihydrochloride (3-chloro-7-methoxy-9 (1-methyl-4-diethylamino-butylamino)-acridine-dihydrochloride)	500
Acranil (3-chloro-7-methoxy-9-diethylamino-β-hydroxy-propylamino-acridine-dihydrochloride)	> 20
Neo-acranil (3-chloro-7-methoxy-9(2-hydroxyethyl-amino-ethylamino)-acridine-dihydrochloride)	> 20
6-chloro-9-(8-diethylamino-1-methocylamino-2-methoxy-acridine-dihydrochloride monohydrate (SN 12868-4-3)	500
9-(4-dimethylamino-cyclohexylamino-6,7-dimethyl-3-nitro-acridine, dihydrochloride)	100

Of the quinolines tested (Table IV), the antimalarial drug 8-(9-diethylaminonylamino)-6-methoxyquinoline dihydrobromide (SN 167-13) was inhibitory at 50 micrograms per ml.,

and Vioform (5-chloro-7-iodo-8-quinolinol) at 4–5 micrograms per ml. The latter was found to be the most effective of all drugs employed, completely inhibiting all seven strains of *C. albicans*. The other two quinolinol amebicides tested were not so greatly inhibitory. Chiniofon inhibited growth at 1,000 micrograms per ml. DeAlmeida and Lacaz (1946) found this drug effective against *P. brasilensis* in a 1:200 dilution. Diodoquin inhibited the growth of *C. albicans* at 500 micrograms per ml. Because of its insolubility, a finely ground suspension was made in brain heart infusion agar and plates inoculated with radial streaks of the organisms.

TABLE IV

In Vitro Studies of Various Quinolines on the Growth of C. Albicans

Drug	Concentration giving complete inhibition of growth (micrograms per ml.)
Vioform (5-chloro-7-iodo-8-quinolinol)	5
Diodoquin (5,7-diiodo-8-quinolinol)	500
Chiniofon (7-iodo-8-quinolinol-5-sulfonic acid)	1000
Quinine hydrochloride (6-methoxy-α-(5-vinyl-2-quinuclidyl)-4-quinolinemethanol) (SN 359)	> 1000
Pentaquine (8-(5-isopropylaminoamylamino)-6-methoxy-quinoline monophosphate) (SN 13276)	> 10
Endochin (3-n-heptyl-7-methoxy-2-methyl-4-quinolinol) (SN 13421)	> 50
Plasmochin (8-(4-diethylamino-1-methylbutyl-amino)-6-methoxyquinoline hydroiodide) (SN 971)	> 20
8-(9-diethylaminonylamino)-6-methoxyquinoline dihydrobromide (SN 167–13)	50
8-(3-aminopropylamino)-6-methoxyquinoline (SN 1452–4)	> 1000
1-(7-chloro-4-quinolylamino)-3-diethylamino-2-propanol diphosphate (SN 8137–5)	> 1000

A series of naphthoquinones (Table V) was tested. The antimalarial drugs SN 3529, SN 5949, and SN 5094 (M 266) gave promising results, inhibiting at 20 micrograms per ml. or below. Hykinone (menadione sodium bisulfite) inhibited growth of 2 strains of *C. albicans* at 50 micrograms per ml. and of 2 other strains at 100 micrograms per ml. Synkavite (2-methyl-1,4-naphthoquinone diphosphoric ester tetra sodium salt) was tested in brain heart infusion agar because the drug was available to us at that time only in tablet form for which broth was unsuitable. Growth was obtained with all strains

through 100 micrograms per ml. This coincides with the work of Schmidt, et al. (1950), who found this compound completely inert in agar medium.

TABLE V

In Vitro Studies of Various Naphthoquinones on the Growth of C. Albicans

Drug	Concentration giving complete inhibition of growth (micrograms per ml.)
2-hydroxy-3-isoamyl-1,4-naphthoquinone (SN 3529)	10
2-hydroxy-3-(2-methyloctyl)-1,4-naphthoquinone (SN 5949)	6
2-[3-(transdecahydro-2-naphthyl)-propyl]-3-hydroxy-1,4-naphthoquinone (SN 8557)	100
3-cyclohexyl-2-hydroxy-1,4-naphthoquinone (SN 5094 or M 266)	20
2-(3-cyclohexylpropyl)-3-hydroxy-1,4-naphthoquinone (SN 5090)	100
2-[3-(cisdecahydro-2-naphthyl)-propl]-3-hydroxy-1,4-naphthoquinone (SN 12320)	90
Hykinone (Menadione sodium bisulfite) 2 strains	50
2 strains	100
Synkavite (2-methyl-1,4-naphthoquinone diphosphoric ester tetra sodium salt)	> 100

In Vivo Results

Vioform was chosen as the drug for further study against *C. albicans* because the other effective inhibitory agents of our *in vitro* tests, the naphthoquinones, are relatively toxic. Vioform was introduced as an amebicide by Anderson and Koch (1931) and its toxicity has been determined (David, Johnstone, Reed and Leake, 1933). Jadssohn and Pfanner (1945) tested Vioform *in vitro* and *in vivo* against *Achorion quinckeanum* and *Epidermophyton Kaufman-Wolf*, and found it somewhat effective.

A series of six rabbits were inoculated intravenously with *C. albicans*, and the attempt was made to prevent infection by oral administration of Vioform. The organism was dosed at 1 ml. of a 1:1000 dilution, a uniformly lethal inoculum according to Benham (1935). Dosages of the drug were based on the treatment for amebiasis in children (20 mg. per 7 kilograms) in 2 rabbits, and arbitrarily at 20 mg. per kilogram in 3 rabbits. See Table VI.

Three rabbits were given their first dose of Vioform one hour previous to inoculation and two rabbits, 12 hours after; a sixth rabbit, a positive control, was inoculated with a standard dose, but was not drugged. The five rabbits were then dosed every three hours for the first 24 hours and five times per 24 hours until death. The dosage schedule was based on the findings of Knight and Miller (1949) and Haskins, Luttermoser and Brady (1950). It was hoped that by frequent dosing a uniformily high blood-iodine level might be maintained.

TABLE VI

In Vivo Studies on Rabbits Inoculated Intravenously with C. Albicans and Dosed with Vioform

Animal	Oral Dosage	Drug before Inoculation	Total Dosage	Death	Autopsy
#1	20 mg/Kg.	7.5 mg.	every 3 hours for 1st 24 hrs. 5 doses/24 hrs. until death	5 days	Liver-neg. Kidney-pos.
#2	20 mg/Kg.	8.7 mg.	every 3 hours for 1st 24 hrs. 5 doses/24 hrs. until death	6 days	Liver-neg. Kidney-pos.
#3	20 mg/7 Kg.	1.8 mg.	every 3 hours for 1st 24 hrs. 5 doses/24 hrs. until death	7 days	Liver-neg. Rt. kidney-neg. Lt. kidney-neg. Milk-pos.*
#4	20 mg/Kg.	none	8.3 mg every 3 hrs. for 1st 24 hrs. 5 doses/24 hrs. until death	4 days	Liver-neg. Kidney-pos.
#5	20 mg/7 Kg.	none	1.1 mg every 3 hrs. for 1st 24 hrs. 5 doses/24 hrs until death	6 days	Liver-neg. Kidney-pos.
#6	Control	none		6 days	Liver-neg. Kidney-pos.

*On autopsy this animal was found to be pregnant and cultures were obtained from the lactating mammary glands.

All rabbits began showing symptoms by the third day with the exception of No. 3. This rabbit never appeared acutely ill, but stopped eating on the fifth day. All rabbits died within 4–6 days with the exception of No. 3, which survived for 7 days; the control dying on the sixth day. On autopsy all cultures from the kidneys yielded *C. albicans* with the exception

of No. 3. This animal was pregnant and its mammary glands lactating, and cultures of the milk yielded *C. albicans*, though the kidneys were negative. It is interesting to note in this connection that two patients with severe vaginitis following aureomycin therapy, Harris (1950) reported the subsidence of their symptoms after a single dose of estrogen.

In view of the essentially negative *in vivo* results, *in vitro* retestings in the presence of serum suggests one reason why the drug fails to inhibit or kill the organisms in the blood stream or deep tissue. *In vitro* tests were set up using ten per cent rabbit serum in different concentrations of Vioform. All dilutions showed growth through 8 micrograms per ml. though in the absence of serum, the growth of the organisms was inhibited by 4–5 micrograms per ml. Studies are in progress to determine whether higher drug concentrations would inhibit the organisms in the presence of serum. Human serum was tested in the same dilutions as rabbit serum and the results were the same, growth occurring in all concentrations through 8 micrograms per ml.

The effect of Vioform on localized moniliasis was tested therapeutically. Monilia infections of man ordinarily are of this nature and involve accessible body surfaces. Two cases of *C. albicans* pruritis and proctitis, one following aureomycin therapy and the other following terramycin therapy were put at our disposal through the courtesy of Dr. William C. Levin. Both cases were placed on Vioform in a dosage of 250 mg. three times daily, and the results indicate clinical effectiveness. Both cases were without symptoms following two days of Vioform therapy, and have remained asymptomatic for one month.

These and other cases are to be reported in some clinical detail at a later date by Dr. Levin.

Discussion

With the increasing use of the oral antibiotics, resistant fungi such as *C. albicans* have attained new prominence. These organisms serve as a potential source of danger when individuals normally harboring them are treated with oral antibiotics. Other fungi such as Aspergillus (Zimmerman, 1950)

existing in the body as saprophytes may gain similar biological advantage with the suppression of the normal flora by these antibiotics. Campbell, et al. (1950), have noted that various antibiotics in common use actually have a stimulatory effect on some of the pathogenic fungi.

Of the drugs showing inhibitory action against *C. albicans*, Vioform (5-chloro-7-iodo-8-quinolinol) was selected for study *in vivo* because of its relatively non-toxic effect when administered orally. David, et al. (1944), determined blood levels of iodine chemically after administration and found that iodine from Vioform was more consistently absorbed than Diodoquin. Knight and Miller (1949) showed that all of the oxyquinoline drugs are to some extent absorbed as measured by blood-iodine. Haskins, Luttermoser and Brady (1950) using I^{131} found that absorption from the intestinal tract was relatively fast, and that it seems likely that Vioform is absorbed and eliminated as such without any marked degradation in the body. However, their studies indicated that Vioform was rapidly excreted and, in order to maintain high blood levels, frequent dosing was necessary.

The results of limited animal studies have proved disappointing in that Vioform was unable to prevent death in systemic moniliasis. Serum neutralization of the drug is one possible explanation for its ineffectiveness. However, limited clinical trials seem to indicate that this drug may be used effectively in cases of pruritis and proctitis. It is hoped that the drug may also manifest its inhibitory action on other accessible body surfaces.

Another quinoline, SN 167-13 (8-(9-diethylaminonylamino)-6-methoxyquinoline-dihydrobromide) shows some promise as a chemotherapeutic agent, though toxicity may limit its use. Further studies seem to be indicated on the naphthoquinones SN 5949 (2-hydroxy-3-(2-methyloctyl)-1,4-naphthoquinone) and SN 5094 (3-cyclohexyl-2-hydroxy-1,4-naphthoquinone) because of their ability to inhibit in low concentrations. No toxic manifestations have been noted in patients receiving 2 gm. daily of SN 5949 (Wiselogle, 1941–1945). The toxicity of SN 5094 was not found in the literature. SN 3529 (2-hydroxy-3-isoamyl-1,4-naphthoquinone) was inhibitory at 10

micrograms per ml., but this drug is relatively toxic (Wiselogle, 1941–1945).

Conclusions

Vioform (5-chloro-7-iodo-8-quinolinol) was found to be the most promising of forty drugs tested *in vitro* against *Candida albicans*. Two naphthoquinones, SN 5949 and SN 5094, and one quinoline, SN 167-13 show promise in inhibiting *C. albicans in vitro*. Vioform was clinically effective against two cases of pruritis and proctitis caused by *C. albicans*. Vioform was ineffective against systemic infections in rabbits.

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PREMENSTRUAL TENSION—RATIONAL TREATMENT

WILLIAM BICKERS AND MARIBELLE WOODS*

The bleeding phase of the menstrual cycle rarely appears unannounced. Premonitory signs and symptoms which herald its approach are experienced by many women. Anxiety states, depression, low abdominal pain, lumbar pain, thirst, breast fullness, and personality changes of a mild anti-social nature announce the approaching guest. These symptoms are accepted philosophically by most women but there is a minority group, perhaps 25%, who require medication. The syndrome has long been recognized. Hippocrates ascribed the symptoms to "agitated blood" seeking its channel of escape at the uterus. Police records of Paris from the early decades of this century show a striking increase in criminal acts of women during the premenstrual week. Menorrhalgia has been suggested by Hamblen as a descriptive term becoming this symptom complex.

Incidence

The symptom complex of premenstrual tension is most often seen in women between the ages of 25 and 35 and only occasionally in younger women. It is most common in the nulligravida but does occur in patients who have had repeated pregnancies. Women with normal cycles of 25 to 35 days suffer more often than those with irregular cycles. A long delayed menstruation is occasionally preceded by ever rising tension but this is the exception. The symptom-complex probably develops only in ovulatory cycles and this may explain the relative absence of prodromal symptoms in patients with oligomenorrhea and polymenorrhea associated with ovulatory failure.

The medical records of an industrial plant employing approximately 1,500 women have shown that 36% of their female employees seek some type of sedation during the premenstrual week. Often the symptoms are a counterpart to

*From the Medical College of Virginia. Presented at the Obstetrics Conference, The University of Texas Medical Branch, Galveston, March 23, 1951.

other gynecological complaints but in the private practice of gynecology about 5% of patients present it as their major complaint.

Etiology

There can be little doubt that the symptoms of premenstrual tension are related to abnormal water metabolism. In 1933, Thomas reported two cases of unusual edema recurring cyclically during the premenstrual period. Associated with the edema were severe headache, coma, abdominal fullness, leg pains and other classical symptoms which are now grouped under the clinical diagnosis of premenstrual tension. Thomas observed that all these symptoms disappeared following the diuresis which occurred at the time of menstruation. Greenhill and Freed first called attention to the frequency of the syndrome and related it to abnormal water retention. Many theories have been advanced to explain the physiologic mechanism of this abnormal water storage, none of them is completely satisfactory.

Frank suggested that the water retention might be due to high estrogen levels because of lowered renal excretion of this hormone. It is known that the ovarian steroids favor a positive water balance. Morton in a recent review of 29 cases places the blame on defective luteinization of the corpus luteum. Israel likewise surmizes a relative hyperestronemia. Estrogen deficiency has also been proposed and treatment with the estrogens recommended. The Biskinds postulate a deficiency of the Vitamin B Complex with consequent impairment of the liver in its role as the detoxifier of the estrogens.

Recently the Smiths have given 20th century confirmation to the 10th century theory of a menstrual toxin. The medieval belief that menstruating women should avoid the vineyards less the fruit fall from the vine now receives scientific support. It has been shown also by Macht that menstrual blood blights the growth of seedling plants. This toxic agent in menstrual blood has been isolated. It is a globulin fraction found in the catabolic end products of endometrial break down. When this substance is injected into rats a tissue edema follows, in larger doses it is lethal to the animals. The toxin

which results from the break down and shedding of endometrium is most abundant where endometrial growth is well developed such as that occurring in the ovulatory cycle. Sudden withdrawal of corpus luteum support is followed by spiral end artery constriction and necrosis of the endometrium. Since menotoxin is present in all menstrual discharge one may speculate on why all patients do not exhibit the signs and symptoms of premenstrual tension. There must be some other factor operating to induce abnormal water storage in those patients in whom the premenstrual symptoms are exaggerated.

The syndrome of premenstrual tension can be reproduced in part during the corpus luteum phase of the cycle, by the daily administration of Pitressin, the vaso-pressor substance of the posterior pituitary. In a group of 16 patients the syndrome was reproduced clinically and pathologically by the daily administration of Pitressin in a dose of 10 units daily. Water storage up to 4 lbs. was artificially induced in some of these patients.

A recent clinical study on 22 patients with severe premenstrual tension showed an average premenstrual weight gain of 6.8 lbs. while normal women with no premenstrual symptoms gain less than 2.5 lbs. In this group the intensity of symptoms varied directly with the amount of weight gained and this paralleled closely the volume of water lost during the first day of menstruation. There can be little doubt that the essential pathologic physiology involved in the production of this syndrome was water retention. In these 22 patients water loss without treatment during the first 24 hours of menstruation averaged 3,270 cc. while asymptomatic patients excrete about 1,700 cc. Premenstrual tension is essentially a water toxemia. The endocrine disturbance leading to this deranged water metabolism is yet unknown.

Pathology

The pathology associated with the premenstrual tension syndrome is edema, vascular congestion, and hyperemia. The edema can be demonstrated on endometrial biopsy.

The endometria of patients with premenstrual tension shows a progestational type. In a study of 22 patients proliferative

phase endometrium was not seen in association with the syndrome. In this group of patients with very severe premenstrual tension endometrial biopsies were taken on 12 at the height of their symptoms. This was usually on the day before onset of flow, but occasionally on the day of flow. The stroma edema was out of all proportion to that normally seen in the immediate premenstrual period. The star and spindle cells of the stroma were separated by large intercellular spaces filled with fluid. In some cases the fluid tissue spaces appear to actually impinge on the glands.

It has been suggested that free peritoneal fluid increases in association with the premenstrual syndrome. Aspiration of the cul-de-sac at the time of laporotomy in patients suffering severe premenstrual tension may reveal in excess of 20 cc. of free intraperitoneal fluid. This fluid on chemical examination proves to be a simple transudate. In severe cases occult edema may go on to obvious clinical edema where pitting over the tibia can be demonstrated.

The recurrent edema associated with this syndrome may be a precursor to chronic vascular congestion and hyperemia which may go on to fibrosis. Taylor has described this state as "congestion fibrosis syndrome." In premenstrual tension the edema always occurs cyclically and completely disappears at the onset or within the first 24 hours of menstruation. It is conceivable, however, that these changes recurring sufficiently often over a protracted period may induce connective tissue hyperplasia in the reproductive organs.

The Clinical Syndrome

In the group of 22 patients under discussion the typical patient was a woman between the ages of 25 and 35 years who complained of distress during the premenstrual epoch and was relieved at or near the onset of flow. Headaches, nervous irritability, insomnia, abdominal bloatiness, low back pain, pelvic fullness, breast tenderness, and unusual thirst were the symptoms most frequently emphasized. In most patients the symptoms began about 10 days prior to menstruation but in others the duration of symptoms was shorter. In

these patients with severe premenstrual tension the average duration of symptoms prior to menstruation was 8.9 days. Symptoms grew progressively worse and reached a crescendo just before or during the first few hours of menstruation. Patients with oligomenorrhea, cycles exceeding 40 days, may enter a long period of progressively distressing symptoms.

In this study patients with symptoms of severe grade were shown to have an average weight gain of 6.8 lbs. while another group with milder symptoms averaged 3.7 lbs., the asymptomatic patient gaining less than 2.5 lbs. There are reports on patients with disabling symptoms who have gained up to 14 lbs. The largest gain in this series was 11.1 lbs. The severity of symptoms paralleled the weight gain. The entire weight gain was lost during the first 24 hours of menstruation in a diuresis which might reach 4,500 cc. The average urine output in the first 24 hours of menstruation in these patients with severe symptoms was 3,270 cc. In the group with milder symptoms and of shorter duration the average output was 2,100 cc. and in asymptomatic patients it is around 1,700 cc.

The menstrual cycle was usually regular in these 22 patients. There were 18 who menstruated on a cycle of 25 to 35 days and there were 4 who had oligomenorrhea with cycles varying from 40 to 82 days. In these cases the symptoms of premenstrual tension began some 3 to 4 weeks before the onset of flow, gradually built to a peak until about the time of the onset of flow at which time diuresis occurred and symptoms were relieved. The premenstrual tension syndrome was not seen in a short cycle. Usually the patient did not complain of severe dysmenorrhea. Although the syndrome of premenstrual tension and dysmenorrhea may appear concurrently it is the exception and not the rule.

Basal temperature charts kept on these patients invariably showed a marked biphasic shift. The basal temperature often rose to 98.4 in the morning of the premenstrual week. Other reports describe faulty or incomplete evidence of progesterone activity as demonstrated by temperature curves. This was not the experience in this group.

Treatment

The patients in this study group suffered symptoms of unbearable proportion through which they could not pass without serious disruption to their normal lives. Many women have mild symptoms which may be treated expectantly.

Symptomatic Treatment

It may be pointed out to the patient that a certain amount of discomfort during the premenstrual period is to be expected and the premonitory signs of normal menstruation can be ignored. For the depression that frequently occurs small doses of amphetamine sulfate have been found effective. Recently this drug has been combined with a sedative and the combination found useful in symptomatic treatment. Analgesics such as aspirin are helpful. Breast symptoms are relieved in part by properly fitted brassieres which elevate the breasts promoting lymphatic and vascular drainage. A saline cathartic taken 1 or 2 days before the expected menstruation often aids in relieving the symptoms.

Hormone Treatment

If it be assumed that the symptoms are the result of unopposed estrogen, it would be rational to treat these patients either by the administration of progesterome during the last half of the cycle or with chorionic gonadotropin in the hope of stimulating corpus luteum activity. Both of these treatments have been used extensively and reported upon in the literature. Progesterone may be administered in the form of injected progesterone or oral progesterone. Anhydrohydroxy progesterone has also been used. The minimum therapeutic dose for either of these hormones given orally is 80 mg. daily. If they are to be employed, treatment should be started ten days before expected menstruation and carried up to the day of flow. The cost of these progestational hormones is high and the therapeutic value certainly is not in proportion to their cost. Chorionic gonadotropin has been administered in a dose of 500 mg. daily beginning on the 10th day before

expected menstruation with only equivocal results. Testosterone in doses of 25 mg. subcutaneously or methyl testosterone in the form of buccal tablets, 10 mg. three times daily during the premenstrual epoch has been extolled in many reports.

The experience of many is that the hormones are without value in the management of the syndrome. From that which is known about the pharmacology of these steroids as related to water metabolism they should exaggerate rather than relieve symptoms. It is difficult to see how either estrogen, progesterone, or testosterone could be effective. Since all of these steroids tend to fix sodium in the tissues and thus favor water retention, it would seem that symptoms would be exaggerated rather than diminished. This seems to be the case.

Vitamin B Complex Treatment

Vitamin B complex has been recommended in treatment of premenstrual tension on the assumption that the syndrome is the result of high blood estrogen secondary to liver damage resulting from vitamin B deficiency. Much doubt has been cast upon the value of this treatment since it has been shown that patients with severe hepatitis have no impairment of estrogen metabolism. Patients with advanced cirrhosis of the liver do not develop these clinical symptoms thought to be related to high blood estrogen. It is true that the administration of vitamin B complex in large doses may improve the general well-being but it apparently has no effects upon tissue hydration which is the essential feature of premenstrual tension.

Protective Globulin Treatment

This has been used experimentally but is not available for clinical use.

Dehydration by Ammonium Chloride and Salt Restriction Treatment

Greenhill and Freed have recommended dehydration of these patients during the premenstrual period by a salt restricted diet and the administration of ammonium chloride in a dose of 1 gm. four times daily. In the group of patients

under study this treatment was administered to 16 patients with severe premenstrual tension. Some found it difficult to tolerate the large doses of ammonium chloride even when the drug was administered in enteric coating. The 16 cases had an average weight gain before treatment of 5.2 lbs., a group that could be classified as the severe type. Treatment was instituted 5 to 8 days before expected menstruation and maintained daily until onset of flow. Strict adherence to this regime produced only partial relief of symptoms in these patients. The average gain for the treated group, the weights being recorded usually on the day before menstruation, was 4.3 lbs. This was some reduction from 5.2 lbs. in the pretreatment cycles. Most of the patients felt that the relief obtained was insufficient to justify the inconvenience of repeated treatments in subsequent months. All of them complained about the salt restricted diet and the majority complained of gastric irritation from the large doses of ammonium chloride.

Dehydration With Pyrilamine 8 Bromo Theophyllinate Treatment

In this group of 22 patients with severe premenstrual tension dehydration with a new drug, Pyrilamine 8 Bromo Theophyllinate, was attempted. The majority of patients in this group were partially or totally incapacitated during the premenstrual week. There were 5 who were bedridden for 1 to 3 days before menstruation. All of these patients had a complete physical examination and medical history. Patients with psychopathic personality, neurosis, and medical disease of any type were excluded. The 22 cases under consideration were, so far as could be determined, patients whose sole complaint was premenstrual tension. Headache, breast pain, backache, nervous tension, abdominal pain, and emotional instability were the major symptoms. Patients with premenstrual tension of a relatively severe degree such as that seen in this group present a major clinical problem.

The 22 patients in the clinical study had previously received some form of treatment with more or less disappointment. Most of them had been on ammonium chloride and salt

restricted diet with only partial relief. All of them were observed for a minimum of one month without any treatment during which time weight was recorded through at least one complete cycle. Without treatment the average weight gain for the group during the premenstrual week was 6.8 lbs., the largest individual gain was 11.5 lbs., and the smallest was 4.2 lbs. An attempt was made to record the intake and output of fluid during these cycles but the inevitable error arising from the patient's failure to accurately note her fluid intake and output makes the results of doubtful value. On the basis of the patient's records the average urine output in the first 24 hours of menstruation was 3,270 cc. The patients were given tablets of Pyrilamine 8 Bromo Theophyllinate, 50 mg. to each tablet, and instructed to take 1 of these tablets 3 times a day beginning 10 days before menstruation. Medication was stopped at the first sign of menstruation. The average weight gain for the group of 22 patients after treatment was 1.4 lbs. More than half the patients were followed in the succeeding cycle with the same treatment and the average weight gain for the group was found to be 1.6 lbs. Eight patients in the series agreed to record their weight during a third cycle without treatment. The average weight gain for this group in the untreated cycle was 6.9 lbs. and accompanying it all symptoms recurred. Although the figures on urine output are not too reliable the 22 patients reported an average output during the 24 hours of menstruation following the treated cycle of 1,450 cc. This stands in contrast to the 3,270 cc. which the same group reported during a control cycle in which no treatment was given. This indicates that the drug is highly efficacious in preventing water storage. Symptomatic relief closely paralleled the smaller weight gain during the treatment cycles. The symptoms of headache, bloatiness, abdominal pain, and nervous tension were significantly reduced in practically all of the patients. In the untreated cycle in which 8 patients co-operated following their treated cycles all the symptoms recurred.

There is no permanent alteration in water metabolism nor is clinical relief obtained except during the period of treatment. In some patients not included in this series treatment

was instituted after the symptoms of premenstrual tension had developed. A diuresis of some degree invariably followed in these patients and most of them were relieved in part or in whole of their premenstrual symptoms.

Study of Water and Chloride Metabolism on Rats

When rats are fed measured amounts of water and placed in a metabolism cage it is possible to recover 104% of the administered water during a four hour collection period, water itself being something of a diuretic. When Pitressin in a dose of 0.5 unit per rat is administered s.c. to the animal 37% of the administered water is recovered in 4 hours. To measure the diuretic effect of a drug to be employed in premenstrual tension it is satisfactory to induce water storage in an animal by the administration of Pitressin, employing a modification of the technic described in the British Pharmacopea. This method simulates the water retention of the premenstrual tension. When Pyrilamine 8 Bromo Theophyllinate in a dose of 0.1 gm./kg. orally is administered to the rat along with 5 cc. of water per 100 gm. of body weight and Pitressin in a dose of 0.5 unit s.c. the anti-diuretic effect of the Pitressin is more than neutralized as is shown by a recovery of 92% of the administered water in the 4 hours as compared to 37% recovery when water and Pitressin alone were given. When the experiment on rats was repeated using ammonium chloride in a dose of 0.1 gm./kg. instead of the Pyrilamine Bromo Theophyllinate compound, only 34% of the administered water was recovered in 4 hours, essentially the same as if water Pitressin alone were given.

These figures were obtained from a series of experiments and in each experiment 4 rats in a single cage were employed. Water in the same volume, 5 cc./100 gm. body weight was used in a group of rats who also received 0.5 unit of Pitressin s.c. and testosterone i.m. The hormone was administered as testosterone propionate in a dose of 5 mg. i.m. given at 4 P.M, on the day preceding the test and repeated at test time on the following day. Water recovery in this group of animals was 23%.

Chloride excretion, as measured by the Volhard-Arnold method, was studied in each group of animals along with the quantitative determination of water recovery. Those animals who received water alone excreted an average of 24 mg. of total chlorides per cage of 4 rats in 4 hours. The animals that received water and Pitressin excreted 68 mg. while those to whom water, Pitressin, and ammonium chloride was given excreted 82 mg. (figure uncorrected for chloride administered). The cages containing 4 rats treated with water, Pitressin, and testosterone yielded an average of 52 mg. of total chlorides. The cages containing rats who received water, Pitressin, and Pyrilamine 8 Bromo Theophyllinate yielded an average of 102 mg. (See table.)

Study of Water and Chloride Metabolism on Humans

Three hospitalized young women volunteered to co-operate in a study of water and chloride metabolism. These patients were aged 22, 27, and 32, respectively. They were patients who had been subjected to no surgical trauma and who had no serious medical or surgical illness. They received no food or water intake after 9 P.M. On the following morning each patient was given 1,000 cc. of tap water orally over a period of 30 minutes. At the same time each patient received 10 units of aqueous Pitressin subcutaneously. The bladder was emptied by catheterization before taking the water and the catheter left in place and connected with a bedside bottle. The urine was collected over a period of 4 hours. During this period the patients excreted 106 cc., 275 cc., and 210 cc., respectively. The 3 patients were then allowed a regular diet until the evening and at 9 P.M. all food and fluid was withheld. On the next morning each of the three patients received 1,000 cc. of tap water orally and 10 units of aqueous Pitressin subcutaneously. In addition each was given Pyrilamine Bromo Theophyllinate in a dose of 100 mg. orally during the period of water ingestion. The dose was repeated 2 hours later and urine was collected from an indwelling catheter over a 4 hour period. The urine excreted was 950 cc., 980 cc., and 1,200 cc., respectively, for each of the 3 patients. It is apparent from these figures that Pyrilamine Bromo Theophyllinate was able

to neutralize almost completely the water storage effect of Pitressin. (See table.)

Table of Water and Total Chlorides Excretion

THE MALE RAT

4 hour test period

Water—5 cc./100 gm. body weight, orally.
Pitressin—0.5 unit/rat, s.c.
Testosterone Propionate—5 mg. at 4 P.M. day preceding test, and again at test time, i.m.
Ammonium Chloride—0.5 gm./kg. or 0.1 gm./kg., orally.
Pyrilamine 8 Bromo Theophyllinate—0.1 gm./kg., orally.

	Average % Water Recovery	Average Total Chlorides per Cage of 4 Rats
Water	104	24 mg.
Water, Testosterone	112	28
Water, Ammonium Chloride (0.1 gm./kg.)	91	..
Water, Pyrilamine 8 Bromo Theophyllinate 0.1 gm./kg.	136	68
Water, Pitressin	37	68
Water, Pitressin, Testosterone	23	52
Water, Pitressin, Ammonium Chloride 0.1 gm./kg.	32	82
Water, Pitressin, Ammonium Chloride 0.5 gm./kg.	75	73
Water, Pitressin, Pyrilamine 8 Bromo Theophyllinate 0.1 gm./kg.	92	102

THE HUMAN FEMALE

4 hour test period

Water—1,000 cc./patient.
Pitressin—10 units/patient, s.c.
Pyrilamine 8 Bromo Theophyllinate
100 mg. at 8:15 A.M. day of test,
100 mg. again at 10:15 A.M., orally

Total Water Recovered Water, Pitressin		Water, Pitressin, and Pyrilamine 8 Bromo Theophyllinate	
Patient 1.	105 cc.	Patient 1.	950 cc.
Patient 2.	275 cc.	Patient 2.	980 cc.
Patient 3.	210 cc.	Patient 3.	1200 cc.

Conclusions

Premenstrual tension is a symptom-complex related to abnormal water storage during the premenstrual period. It is essentially a water toxemia. Intensity of symptoms vary directly as the amount of water retained. Relief of symptoms accompany the diuresis which usually occurs on or about the

first day of menstruation. Symptoms are severe when premenstrual weight gain exceeds 5 pounds. The etiology of the disturbed water metabolism is unknown but appears to be related to some Pitressin-like toxin.

In a group of 22 patients with severe symptoms the average weight gain was 6.8 pounds. Symptoms were prevented when the weight gain was prevented by blocking the water fixing toxin of the premenstrual period. The most effective agent for blocking the water storage was found to be Pyrilamine-8-Bromo-Theophyllinate.

Pitressin induced edema in rats is not influenced by testosterone or ammonium chloride but is unblocked by the Pyrilamine-8-Bromo-Theophyllinate compound. Pitressin induced edema in 3 human females was effectively unblocked by the Pyrillamine-8-Bromo-Theophyllinate compound.

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FRAGILITY OF *TETRAHYMENA GELEII* IN DIFFERENT SALT CONCENTRATIONS

IBEN BROWNING, NONA B. VARNEDOE AND LAURALEE R. SWINFORD*

Many instances of anomalous population decreases of protozoa have been published. A common occurrence of this sort is the decrease in numbers of protozoa found at the beginning of a growth curve after inoculation into a different kind of medium (Browning, 1949, for example). Phelps (1947) reported the isolation of "Strain K" of a Tetrahymena—like ciliated protozoan which has a very unusual rate of change in numbers. The logarithm of the number of organisms graphed against time produced a growth curve which looked like a sine curve superimposed on a slope with an "average" generation time of about 3 hours. This curve indicated an actual decrease in the number of organisms between population "surges."

In an investigation involving different salt concentrations in *Tetrahymena geleii* cultures, populations were seen to decrease on occasion during the exponential growth phase. A methodical investigation was made to determine the cause of decrease.

Materials and Methods

The organism used in these experiments was ***Tetrahymena geleii*** (Thomas-Phelps strain). The medium used was modeled after that used by Phelps (1935), except that one per cent Proteose-Peptone was substituted for yeast extract.

The above medium is hereafter referred to as the "control medium." The other medium used in the experiment had all of the salts increased fifty-fold, with the Proteose-Peptone and

*From the M. D. Anderson Hospital for Cancer Research, Houston, Texas. Received for publication April 2, 1951. Since this paper was completed, it has come to the authors' attention that, especially in Warburg experiments, the effect of fragility of these organisms is often important. In optimal medium (particularly with respect to calcium), a small fraction of the organisms is destroyed whereas in other media, up to 95% may be destroyed. Details of this work will be published at a later date by Dr. W. J. Wingo of this institution.

water remaining the same. The latter solution is called "50x medium."

Throughout the experiment, a temperature of 28.14 ± .05°C., a low light intensity and a pH of 6.8 to 7.2 were maintained in cultures of Tetrahymena.

In the case of 50x medium, autoclaving was found to produce a considerable lowering of the pH, but adjustment either prior or subsequent to autoclaving was feasible. Normal medium was sterilized by autoclaving and no appreciable pH change was produced in it.

When the population of organisms reached a fairly high number in certain of the cultures, the flask (250 ml. Erlenmeyer) was mounted on a shaker and was oscillated at a speed of approximately one cycle per second in reciprocal motion through approximately one and one-half inches. Shaking was accomplished also by gently swirling the flask by hand so that the organisms were more uniformly distributed for sampling purposes.

With the exception of populations having very low numbers, more than one thousand organisms per sample were counted in a Sedgwick-Rafter chamber. One drop of mercuric chloride solution was added to each two milliliter sample as a killing and fixing agent. The effect of this fixative was studied by comparison of numbers of organisms per milliliter in a given aliquot immediately upon sampling and again forty-eight hours later.

The mercuric chloride fixative was suspected of insufficient preservation of organism; therefore, tests were made to determine the degree of deterioration permitted during periods following fixation.

A series of fixatives was tried and found to be faulty in some respect. The most satisfactory is: 1/20 ml. formalin per ml. of medium with organisms to be preserved. Although clumping may be induced in Tetrahymena so preserved, it may easily be prevented or broken up by stirring. Such clumping is caused by the interlocking of cilia of the preserved organisms. No decrease in numbers of organisms preserved with formaldehyde was found in four days following fixation, and the gross and nuclear morphology remained entirely clear.

Data and Results

Normal growth characteristics of the organisms used are shown in Figure 1, curve 1. Curve 2 in Figure 1 was high at the beginning because of a massive inoculum from a culture

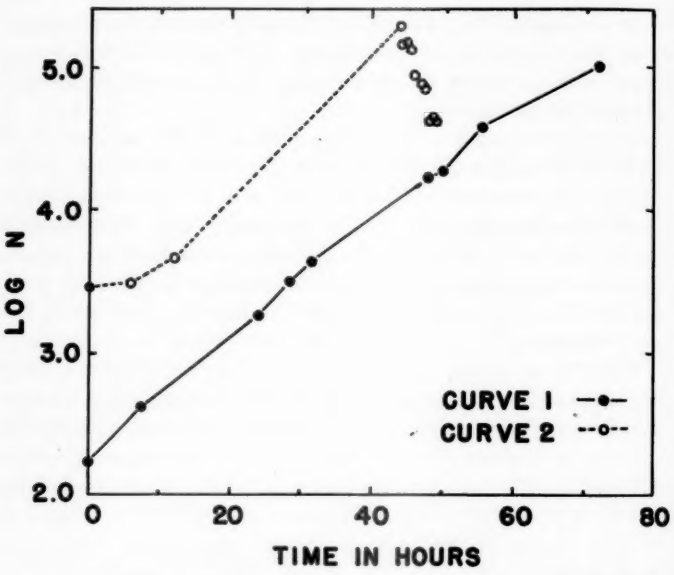

FIG. 1. Curve I represents a culture grown in normal medium—the control. Curve 2 represents a culture of Tetrahymena in medium containing a salt concentration 50x normal. Its rapid decline is attributed to shaking (upon sampling) at 15 minute intervals.

in the late exponential phase of growth. Curve 2 is a typical expression of the change of number of organisms present in 50x medium after very long (sixteen hours) and very short (fifteen minutes) sampling intervals. The shaking of a flask preparatory to taking a sample with a siphon is shown by this curve to have destroyed approximately 50% of the population. This is at variance with the following table in that almost twice as many organisms proportionately were destroyed.

Table I shows a comparable shaking treatment of normal and 50x medium with accompanying determinations of numbers of organisms.

TABLE I

Remarks	Normal Medium	50x Medium	Remarks
Beginning count	40,800	19,231	Beginning count
Shaken once per minute for 15 minutes	39,500	13,940	Shaken once per minute for 10 minutes
Population decrease	3.2%	27.4%	

The disappearance of cells was consistent only as a qualitative phenomenon. It has not been made quantitative. Some of the reasons for this are listed below:

1. When 50x medium was autoclaved, an initial pH 7 was decreased by 1 to 1.5 units. The lowering of pH was accompanied by the appearance of varying amounts of precipitate, thus the medium was altered in chemical constitution.

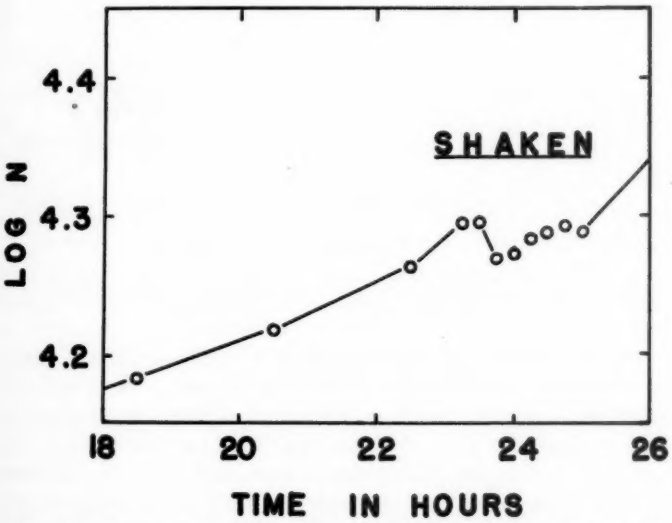

FIG. 2. This curve represents organisms grown in medium with 50x salt concentration. Continuous shaking with samples taken as shown affected the total population and altered the slope normally expected. This curve represents the minimum effect found in this series.

2. Readjustment of pH had to be accomplished with sterile NaOH, therefore varying amounts of Na^+ ions had to be added.

3. If pH were initially set quite high (pH 8 to 8.2) a still different amount of precipitate was produced upon autoclaving.

Figure 2 is a growth curve which shows a minimum, but definite destructive effect on the cells by shaking of the culture flasks.

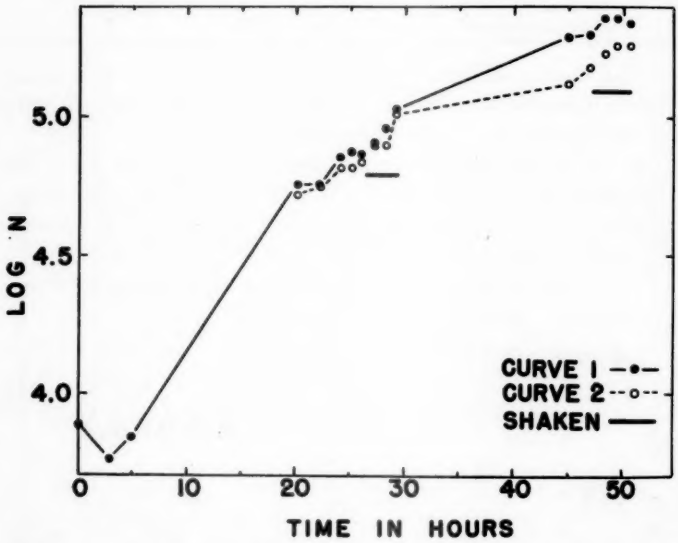

FIG. 3. Curve I represents organisms grown in normal medium, killed with mercuric chloride and counted immediately. Curve II shows aliquots of the same samples counted 48 hours after preservation.

Figure 3 shows an experiment which was done to determine whether mercuric chloride, which was used as a fixative in these experiments, could have accounted for the decrease in numbers of organisms by improper preservation. It will be noted on the accompanying graph that a count of the number of organisms which was delayed 48 hours shows a significant decrease in numbers. The decrease of organisms increases toward the end of the curve, in Figure 3, and others which were done on the same basis.

Figure 3 also demonstrates that populations between 50 and 100 thousand showed no significant effect of continuous shaking compared with shaking for the purpose of sampling.

Conclusions

A decrease in numbers of organisms was found either upon shaking mechanically after a long period of quiescence, or upon simple swirling of the culture flask in order to obtain a more homogeneously distributed sample. The decrease was consistently found in media containing 50x salt concentrations, but was not found to any great extent in normal medium. There was no *consistent* quantitative decrease in numbers of organisms in the cultures under study.

The criterion of number of organisms present was observation in a Sedgwick-Rafter counting chamber, therefore the criterion of destruction was necessarily non-recognition. Some debris was always found in cultures showing a sequence of decreasing numbers, and must have been produced by the destruction of the Tetrahymena. Destruction by gentle shaking indicates that each organism destroyed had an unusually weak cortex.

An alternative explanation for the decrease in numbers was considered. It was thought that samples counted in sequence over a relatively long period of time might show a decrease of numbers due to improper fixation by mercuric chloride. This possibility was ruled out by counting the various samples out of sequence; for example: the first sample first, last sample second, middle sample third, etc., and all within a very few hours. Essentially the same decrease was found by this method as by sequence counting.

The establishment of the phenomenon of cellular fragility is in itself a very small item. It has considerable significance, however, with respect to its implications concerning experimental procedures. The fact that Tetrahymena shows a tendency to break up in an unusual concentration of salts that it normally requires suggests the possibility that this cell might break up in any unusual environment. This has been found to be true in otherwise normal medium of high osmotic pressure. (Browning, 1949.)

One of the experimental requirements for future work with this organism will be, therefore, the demonstration that the population is not primarily affected by fragility. This would apply in particular to experiments involving population determinations, or respiration measurements dependent on populations. Since a cell which has disintegrated leaves all of its physical constituents in the medium, yet cannot reproduce, photometric measurements of populations would be in error to the extent that fragments are mistaken for living organisms. Browning (1948) reported the appearance of large amounts of gelatinous material in media having high osmotic pressure, so that these media became "milky" in appearance and the procedure of counting became prohibitively difficult after 42 hours because of accumulated debris. In this instance, photometric measurements would have been completely meaningless.

Breaking of the organisms due to sampling might easily lead to an erroneous conclusion regarding the meaning of organism counts. If samples were taken at regular intervals of three hours, for example, there would be breakage and subsequent recovery of the population to and beyond the initial number so that the next sample would indicate an increase in population. This could completely hide the fact that a significant percent of the population was destroyed by the previous sampling procedure. A sequence of such evenly timed samples could lead to the conclusion that a given culture has a long generation time, when it might actually have a short generation time, and be quite fragile. In order to detect or prevent such an error, one should make counts from samples taken at irregularly spaced intervals. Fragile organisms should be suspected if frequent sampling tends to establish a longer generation time than infrequent sampling.

It would appear in the light of these findings, that, in experiments using medium which approaches the limit of chemical or physical tolerance of Tetrahymena, the maximum accuracy in the determination of generation time (rate of growth) would be obtained by a minimum number of well established points. It is suggested that with these cells, experiments involving shaking and/or unusual medium for a limited period of time

should take into account the number of intact cells at both the beginning and the end of the experiment.

Summary

A significant percentage of *Tetrahymena geleii* (T-P) was demonstrated to disintegrate upon agitation of the culture medium provided the medium contained fifty times the normal salt concentration. The best preservative for this organism was found to be five percent formaldehyde. By use of this fixative, organisms can be made to retain their morphology clearly for several days. Varying fragility due to medium is discussed with respect to its effect upon experimental procedures.

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ACID FUCHSIN METHYLENE BLUE AGAR FOR ISOLATION AND CULTIVATION OF *PROTEUS**

JODIE L. EDGE† AND E. STATEN WYNNE‡

Plating media which have been used for the isolation of *Proteus* include Endo agar, eosin methylene blue (EMB) agar, MacConkey agar, Salmonella-Shigella (SS) agar, desoxycholate citrate agar, and the bismuth sulfite medium of Wilson and Blair (Hynes, 1942; Neter and Clark, 1944). Endo agar has proved inadequate due to instability of the dye-sulfite complex in the presence of light (Endo, 1904) and masking of nonfermenting colonies by diffusion of metabolic products from fermenting colonies (Holt-Harris and Teague, 1916). EMB agar, MacConkey agar, SS agar, and desoxycholate citrate agar have not been entirely satisfactory because only lactose fermenters produce colored colonies (Wynne, *et al.*, 1942; Jeter and Wynne, 1949), and weak fermenters may be mistaken for nonfermenters (Lederberg, 1948). Furthermore, lactose-negative organisms may be somewhat inhibited on the EMB medium (Fredericq, 1947). Finally, bismuth sulfite agar has been reported to yield a relatively low percentage of isolations of *Proteus* (Hynes, 1942).

Acid fuchsin methylene blue (AFMB) agar, developed in this laboratory to facilitate differentiation of lactose fermenters and nonfermenters, was reported by Jeter and Wynne (1949) to be superior to EMB agar in the cultivation of stock strains of *Proteus* OX-19 and *Proteus vulgaris*. The present investigation is concerned chiefly with a comparison of AFMB agar, Endo agar, EMB agar, MacConkey agar, and SS agar for the primary isolation of *Proteus* from clinical material and for the cultivation of freshly isolated strains.

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*From the Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma. Submitted for publication March 21, 1951.

†Present address: State Department of Health Laboratories, Oklahoma City, Oklahoma.

‡Present address: Department of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

Experimental

All media except the AFMB agar were dehydrated products of the Difco Laboratories (1948). Streaking of plates was accomplished by spreading a standardized drop of inoculum with a glass elbow rod previously dipped in 95% alcohol and flamed. Urine specimens were streaked undiluted, while material from ear infections was diluted by placing a loopful in 3 ml. of sterile water. With fecal specimens, roughly 1 gm. was placed in a tube of 1% desoxycholate broth, and after incubation for 12 hours a loopful was employed for streaking. Incubations were at 37° C. and, except where otherwise stated, for 24 hours. Colonies suspected of being *Proteus* were identified by biochemical tests given in Bergey's Manual (Breed, *et al.*, 1948) and in the identification scheme of Cook (1948).

Clinical material was obtained from Central State Hospital, Norman, and from the out-patient clinic of the University of Oklahoma Hospital, Oklahoma City. One hundred and fifty

TABLE I

Numbers of Proteus Strains Isolated on Five Plating Media

Source	Medium	P. vulgaris	P. mirabilis	P. morganii	P. rettgeri	Total
150 urine specimens	AFMB	18	8	3	5	34
	EMB	14	3	1	1	19
	Endo	15	3	1	1	20
	MacConkey	15	4	1	3	23
	SS	18	7	2	4	31
	All	19	9	3	5	36
6 fecal specimens	AFMB	4	2	0	0	6
	EMB	3	2	0	0	5
	Endo	3	2	0	0	5
	MacConkey	3	2	0	0	5
	SS	4	2	0	0	6
	All	4	2	0	0	6
2 ear infections	AFMB	0	2	0	0	2
	EMB	0	1	0	0	1
	Endo	0	1	0	0	1
	MacConkey	0	1	0	0	1
	SS	0	2	0	0	2
	All	0	2	0	0	2
All	AFMB	22	12	3	5	42
	EMB	17	6	1	1	25
	Endo	18	6	1	1	26
	MacConkey	18	7	1	3	29
	SS	22	11	2	4	39
	All	23	13	3	5	44

pathological urines, 6 fecal samples from adults with diarrhea, and 2 ear infections were cultured. The number of isolations made from each medium and the species of *Proteus* identified are given in Table I. It should be noted that in no case was more than one *Proteus* species isolated from a given specimen.

The most effective media were AFMB agar and SS agar. Though the former yielded a slightly higher number of positive isolations, the difference was probably not significant. These results are compatible with those of Neter and Clark (1944), who listed the following media in the order of decreasing value for isolation of *Proteus:* SS agar, desoxycholate citrate agar, MacConkey agar, and Endo agar. An experimental sample of dehydrated AFMB agar furnished through the courtesy of the Difco Laboratories, Inc., was tested with representative clinical specimens and found not to vary significantly from the medium prepared from the ingredients.

For evaluation of the above media for cultivation of *Proteus*, 1 drop of an 18-hour broth culture was added to 20 ml. of sterile water. After agitation a standardized loop inoculum was employed and spread with a glass elbow rod as above. Relative merits of the 5 plating media for cultivation of 10 freshly isolated *Proteus* strains are given in Table II. AFMB agar, Endo agar, and MacConkey agar appeared to be of approximately equal value for the cultivation of *Proteus*, while EMB agar and SS agar were decidedly inferior.

TABLE II

Comparative Growth of Proteus on Five Differential Media

Isolate No.	Source	Species	Medium				
			AFMB	EMB	Endo	MacConkey	SS
1	urine	*P. vulgaris*	165	39	177	247	54
2	feces	*P. vulgaris*	226	0	248	263	53
3	feces	*P. vulgaris*	223	168	224	261	104
4	urine	*P. mirabilis*	153	50	135	105	3
5	urine	*P. mirabilis*	79	38	89	100	50
6	feces	*P. mirabilis*	88	0	77	93	6
7	ear	*P. mirabilis*	84	17	83	33	3
8	urine	*P. rettgeri*	32	38	94	12	0
9	urine	*P. rettgeri*	37	22	101	8	1
10	urine	*P. morganii*	100	0	98	75	0
	Total.....		1187	372	1326	1197	274

The figures refer to colonies developing from roughly comparable inocula.

Summary

It is concluded that for general work with *Proteus* involving both isolation and cultivation, AFMB agar is the medium of choice.

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STUDIES ON ANTIBIOTICS AND THE NORMAL INTESTINAL FLORA

SYDNEY M. FINEGOLD*

The introduction of succinylsulfathiazole (sulfasuxidine) and phthalylsulfathiazole (sulfathalidine) in 1941 (Poth and Knotts) and 1943 (Poth and Ross) opened a new field in chemotherapy. Previously no agent had affected the intestinal microflora profoundly and consistently without local or systemic toxic effects. These drugs reduce *Escherichia coli* and *Clostridia* in the gastrointestinal tract to very low numbers or eliminate them completely. This alteration can be maintained during a prolonged course of treatment. These drugs have become important adjuncts in bowel surgery, effective therapeutic agents in various intestinal diseases, and useful research tools in the field of nutrition.

With the advent of the antibiotics, the question naturally arose as to whether some of these agents might not be useful in reducing the number of intestinal microorganisms and treating certain enteric conditions. Penicillin does not seem to offer much promise as an intestinal antiseptic. Streptomycin is very poorly absorbed from the gastrointestinal tract after oral administration, and relatively high concentrations may be obtained in the feces. Unfortunately, its action on three important groups of intestinal organisms (*E. coli*, *Clostridia*, and *Strep. fecalis*) is inconstant, and high degrees of resistance are rapidly developed to the drug, resulting in reversion of counts to pretreatment levels (while treatment is continued) in a large percentage of cases (Poth *et al.*, 1948, and Lockwood and coworkers, 1949). Recently, supplemental agents have been used in an attempt to overcome these drawbacks to the use of streptomycin. Pulaski and Connell (1949) used aluminum pectinate and glucorono-lactone in addition to streptomycin in two small series of cases with gratifying results. A majority of the patients maintained suppressed *E. coli* counts with no

*J. B. Kass Fellow from the Surgical Research Laboratory, The University of Texas Medical Branch, Galveston. Received for publication April 1, 1951.

tendency towards reversion for 16 days. Bacitracin is also largely retained within the intestinal tract on oral administration and profoundly affects *S. fecalis* and the *Clostridia.* Apparently low counts can be maintained for at least two or three weeks on continued treatment (Poth). The optimum dosage has not been established. Chloramphenicol (chloromycetin) affects only *E. coli* among the normal intestinal flora (Pulaski and Connell, 1949). According to Woodward and his group, it may be of value in the treatment of typhoid and cholera. Aureomycin, given orally, effects only a moderate decrease in *E. coli* organisms and does not affect other normal inhabitants of the gastrointestinal tract (Pulaski and Connell, 1949). Recent reports by McVay and Meleney indicate that this antibiotic, as well as bacitracin, may be effective in the treatment of amebic dysentery. Polymyxin is poorly absorbed from the gastrointestinal tract and completely suppresses coliforms in a short period without a tendency for resistance to develop. It is the only agent effective against *Pseudomonas pyocyaneus* (Pulaski, 1949).

The possibility of combining some of the above agents in the hope of producing even greater suppression of intestinal organisms has been partly explored. Penicillin adds nothing to the effect of sulfasuxidine and antagonizes the action of sulfathalidine (Poth, Wise, and Slattery, 1946). The combination of streptomycin and the intestinal sulfonamides gives results comparable to those obtained with streptomycin alone (Poth and Lockwood). Bacitracin and the intestinal sulfonamides were found to be mutually antagonistic (Poth). The combination of polymyxin and bacitracin is currently being evaluated by Pulaski and co-workers.

The present study was undertaken to evaluate a new antibiotic, "circulin" (antibiotic Q19 described by Murray and Tetrault, 1948), as an intestinal antiseptic and to assess the value of combined oral administration of streptomycin (supplemented with aluminum pectinate) and bacitracin. Meleny has reported that the combined use of streptomycin and bacitracin is effective in certain mixed infections even to a degree to suggest a synergistic action.

Materials and Methods

A. Circulin Experiments*

Five mongrel dogs were used as subjects; four dogs received dosages of circulin of 60, 120, 240, and 480 mgms./Kg. body weight/24 hours respectively for 10 to 11 days, and the fifth was given a combination of 240 mgms./Kg. body weight/24 hours of circulin and 1 gram/Kg. body weight/24 hours of sulfasuxidine for 11 days and then sudfasuxidine alone for an additional 23 days. Each dog was put on a diet of ground horse meat for three days before the drug was begun. The drugs were then incorporated in meatballs made from the horse meat so that one meatball contained one-sixth of the daily dose. Each dog was given one meatball every four hours with no feeding in between, so that each meatball (and consequently each dose of the drug) was taken promptly every four hours.

Specimens of feces were obtained by inserting a piece of sterile glass tubing into the rectum and aspirating by means of an attached rubber bulb; these specimens were obtained before the drug was started and then daily during the treatment period. Successive dilutions were made of the specimen of feces in 9 cc. of sterile normal saline so that dilutions of from 10 to 10^{10} were obtained. One cc. of every other dilution (10, 10^3, 10^5, 10^7, or 10^2, 10^4, 10^6, 10^8), so as to cover the range of probable growth, was added to each of four tubes of sodium azide medium (for *S. fecalis*), four tubes of anaerobic (thioglycollate) agar unheated (for anaerobes) four tubes of anaerobic agar heated at 80° C. for 15 minutes after inoculation (for anaerobic sporeformers), and to four sterile Petri dishes, to which desoxycholate agar was then added (for coliforms). Para-aminobenzoic acid was added to the cultures of feces from the dog getting sudfasuxidine to produce a concentration of 5 mgms. per cent. When the specimen of feces was too solid to be pipetted, dilution was made on a weight-volume basis in a sterile bottle or flask containing sterile glass beads to facilitate thorough mixing of the feces and saline.

*Circulin was supplied by The Upjohn Company as a powder with a potency of 2500 units/gram. Sulfasuxidine was supplied by Sharp and Dohme, Inc.

Cultures were incubated at 37° C. for 48 hours and then read. Smears were made from the sodium azide tubes since other organisms grew in this medium, particularly *Actinomyces* and yeasts.

On the third day after treatment was stopped, the four dogs on circulin alone were sacrificed for pathological examination.

B. Streptomycin-Bacitracin Experiment*

Four normal human subjects were used in this experiment. Two subjects, MF and SF, were given 0.33 grams of streptomycin every four hours along with 1.33 grams of aluminum pectinate every four hours for 21 and 26 days, respectively. On the ninth day MF began taking approximately 49,000 units of bacitracin every four hours in addition and received this for 12 days. On the ninth day SF began taking approximately 61,000 units of bacitracin every four hours and continued this in addition to the streptomycin for 17 days; on the twentieth day sulfathalidine was added to the other drugs in the amount of 1.5 grams every four hours, and this combination was maintained for 6 days. The other two subjects, MA and MM, were begun on bacitracin, 50,000 units and 75,000 units every four hours, respectively, and continued for 23 and 24 days, respectively. On the eighth day of this treatment, both subjects received in addition streptomycin and aluminum pectinate in the same dosage as MF and SF; this combination was maintained for 15 and 16 days, respectively.

Bacitracin when taken as a solution had a very disagreeable taste and tended to producec severe nausea; consequently the powder was weighed out and capsuled. The streptomycin was taken sometimes as tablets and sometimes as a solution. The aluminum pectinate was taken in capsules. All of the subjects were placed on a low residue diet three days before treatment began and remained on this diet, with added vitamin supplements, until the conclusion of the experiment.

Stool specimens were collected daily in covered cardboard containers and refrigerated until cultured. Dilutions and cul-

*Streptomycin was supplied as the calcium chloride complex by Merck and Company and as the sulfate by The Upjohn Company. Bacitracin was supplied by The Upjohn Company. Aluminum pectinate was supplied by the Commercial Solvents Corporation.

tures were then made as in the circulin experiment. Para-aminobenzoic acid was added to the cultures when indicated as in the circulin experiment. Also, 0.5% potassium sulfite (0.1 cc. to the 1:10 dilution tube) was added as indicated to inactivate bacitracin and 1:300 fresh hydroxylamine hydrochloride (0.5 cc. to the 1:10 dilution tube) to inactivate streptomycin.

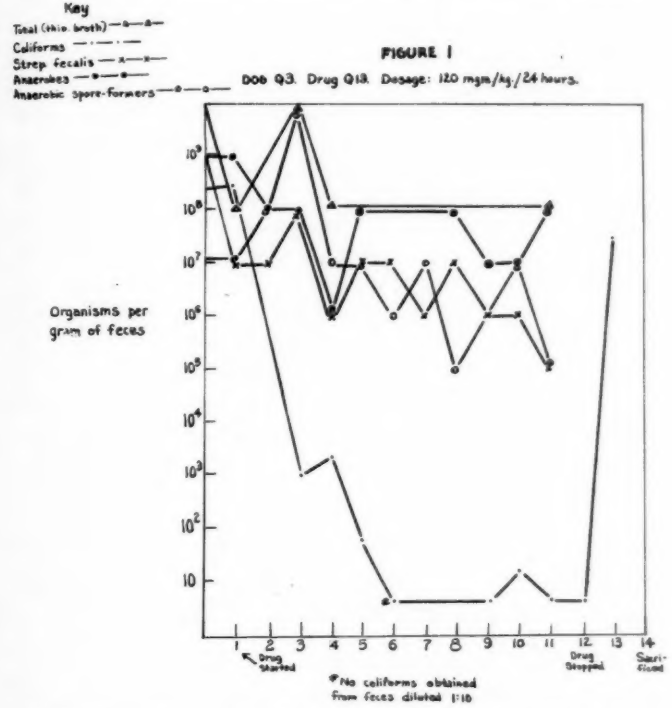
In view of the reported renal toxicity of bacitracin on parenteral administration and the relatively high dosage of bacitracin used, although absorption from the gastrointestinal tract is poor, urinalyses were done on all the subjects before treatment was begun and then at weekly intervals.

Results

With all the dosages used, the only group of organisms affected to any extent was the coliforms. On a daily dosage of 480 mgms. these organisms were reduced over a period of five days to less than ten organisms per gram of feces; however, the count rose rapidly to the pre-treatment level after only two days. With 240 mgms. daily, however, the coliform count reached the same low level within 36 hours and was maintained for 8 days; reversion of the count occurred while treatment was still in progress. On a dosage of 120 mgms. daily (see Fig. 1) the coliform count was gradually reduced, reaching a level of less than ten organisms per gram of feces after five days. The counts remained at approximately this level for another six days, rising rapidly when treatment was discontinued. The coliform count in the dog receiving 60 mgm. daily of circulin was reduced to about 10^2 organisms per gram of feces and after two days began to rise slowly, reaching between 10^4 and 10^5 organisms at the time treatment was discontinued.

The combination of circulin and sulfasuxidine brought the coliform count down more slowly than the circulin alone (about as fast as would sulfasuxidine alone). Furthermore, *Clostridia* were not reduced in numbers (whereas they are greatly and consistently reduced with sulfasuxidine alone) even when the circulin was discontinued. The coliform count began to rise just before the circulin was discontinued and

continued to rise gradually until it reached the pre-treatment level in about three weeks, although sulfasuxidine was continued. Such reversion is not seen with sulfasuxidine alone.

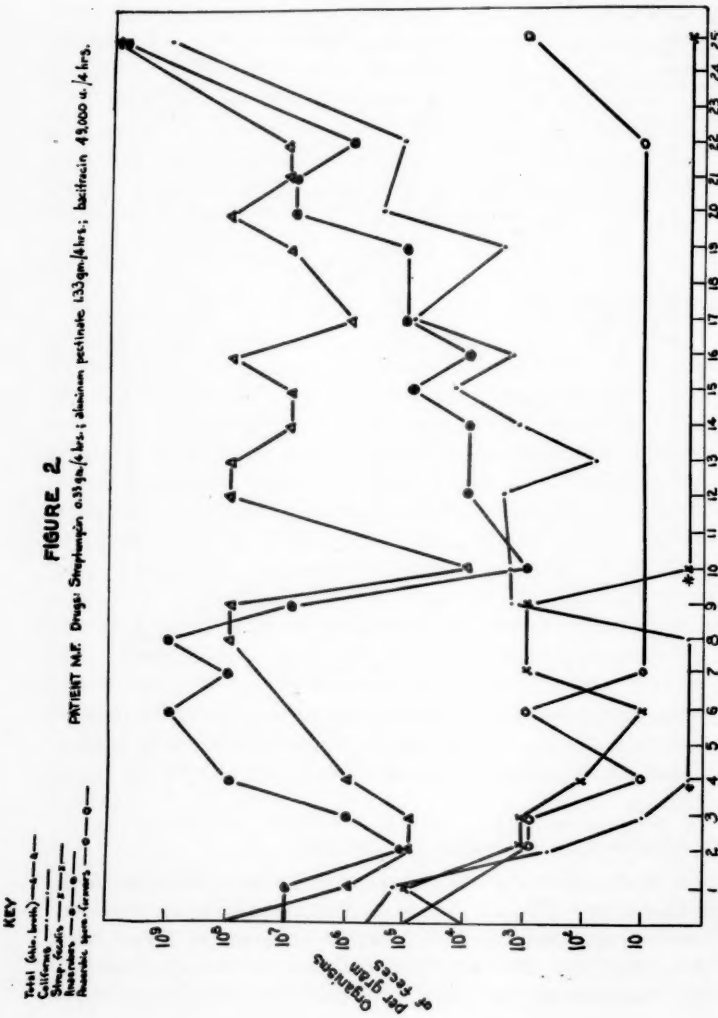


Gross examination of the sacrificed dogs revealed hemorrhage in the bowel wall in the dogs receiving the two highest dosages and a suggestion of mucosal hyperemia in the other two dogs.

B. Streptomycin-Bacitracin Experiment

In the two subjects starting on streptomycin and aluminum pectinate (see Fig. 2), the anaerobic spore-formers were reduced to very low levels (10 organisms/gram of feces) in a week, after first showing much daily variation. These organisms persisted at the above low level after bacitracin (and

sulfathalidine also in one case) had been added. *S. fecalis* showed a decrease in numbers followed by an immediate increase in one case on streptomycin and no response in the



other case. Following the concomitant administration of bacitracin, the counts immediately dropped to less than 10 organisms per gram of feces in both cases and remained at that level for the rest of the treatment period. In both cases, the coliforms dropped to very low levels within three days of the addition of the streptomycin, but then began to rise again rapidly after three or four days. After bacitracin was added, these counts continued to rise more gradually, reaching normal levels in about two weeks. After bacitracin was added to streptomycin, the anaerobes were reduced in number to about 10^3 organisms per gram of feces in both cases. These counts began to rise again slowly just after reaching their lowest point, and they were normal in two weeks. The addition of sulfathalidine to the other two drugs in the one subject appeared to have only a slight temporary effect on the coliform and anaerobe groups, if any.

The two subjects who started on bacitracin (see Fig. 3) responded with a rapid (72 hours) fall in the *S. fecalis* counts to less than 10 organisms per gram of stool, and these counts were maintained throughout the treatment period without change even after the streptomycin and aluminum pectinate were added. In both cases the anaerobic spore-formers were reduced on bacitracin treatment, rose again for several days, and then started to decrease again. With the addition of streptomycin and aluminum pectinate, these counts continued decreasing to 10 organisms per gram of feces and remained at that point for the duration of treatment. In both cases there was a prompt rise in the coliform count to high levels (10^9) following the institution of bacitracin therapy. When streptomycin and aluminum pectinate were added, there was a small decline in the coliform count which was not sustained. The anaerobes were not affected in these two patients.

With the exception of nausea following the ingestion of bacitracin solution (which was eliminated by the use of capsuled bacitracin powder), there was nothing to suggest possible toxic effects from these drugs or their combinations in this experiment. The urinalyses were all entirely normal throughout the treatment period; there was no instance of albuminuria or abnormal microscopic findings. The aluminum

pectinate was responsible for an occasional feeling of bloating when taken near mealtime; the subjects learned that it was necessary to reduce their food intake. Inasmuch as the sub-

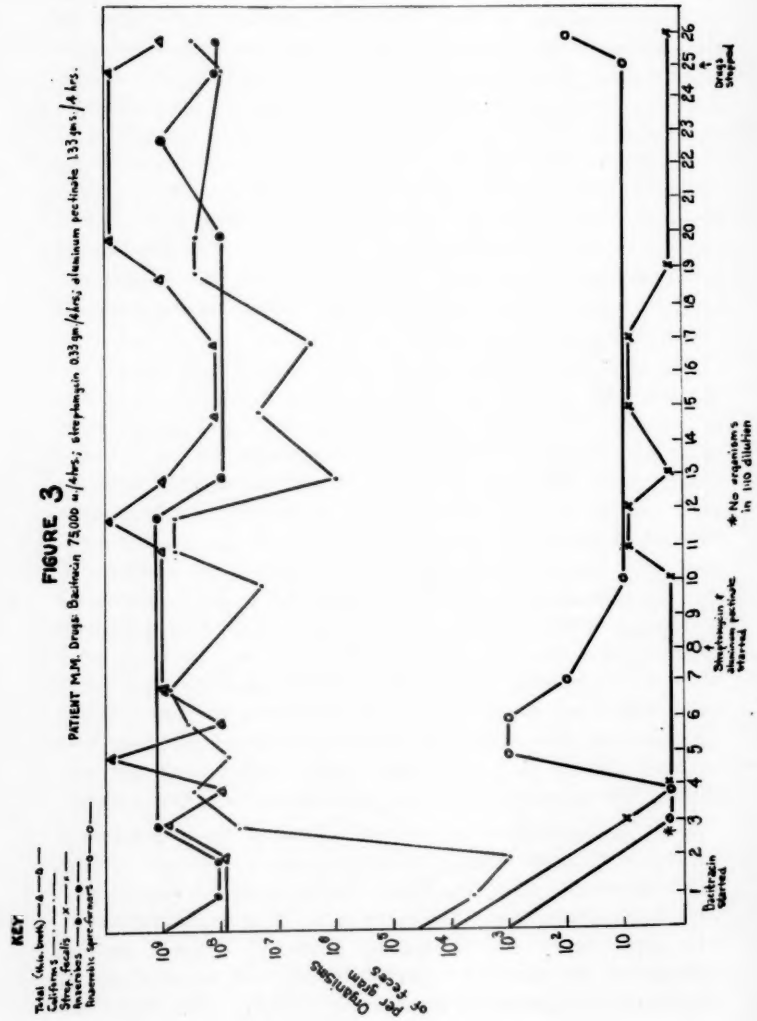


FIGURE 3
PATIENT M.M. Drugs: Bacitracin 75,000 u./4 hrs.; streptomycin 0.33 gm./4 hrs.; aluminum pectinate 133 gms./4 hrs.
KEY
Organisms per gram of feces
Bacitracin started
Streptomycin & aluminum pectinate started
* No organisms in 1:10 dilution
Drugs stopped

jects managed to lose a few pounds of weight during the treatment period in spite of the high carbohydrate content of the low residue diet (and with complete satisfaction of appetite), it is believed that an investigation of aluminum pectinate as a possible aid in reducing would be warranted.

Conclusions

It is obviously impossible to draw valid conclusions from a study involving as few subjects as the present one. Unfortunately limitations of drugs and time necessitated a restricted study. It is felt that these results may prove useful, however, as suggestive evidence which may point the way for additional work.

It is further pointed out that the results in the circulin experiment may be somewhat modified from the true picture in that nothing was incorporated into the media to inactivate the circulin and thus prevent its exerting a further effect on the organisms during the incubation period for culturing the stool specimens. Unfortunately an antagonist for circulin which would not in itself affect the intestinal microorganisms has not as yet been found. In the end, it matters little actually, since circulin shows such little promise. In the dog which showed the best response to the drug, only the coliforms were reduced to low levels. Furthermore, there appears to be a fairly narrow dosage range for effectiveness, above and below which the results are poor. Finally there is suggestive evidence of toxicity even in the lowest dosage used, which was ineffective. The combination of circulin and sudfasuxidine adds nothing to the effectiveness of circulin (and possibly slows its action) and detracts from that of sulfasuxidine.

It will be noted that both subjects on streptomycin plus aluminum pectinate exhibited reversion of originally lowered coliform counts within a few days; hence the above combination seems in this study to be not nearly so effective in preventing such reversions as the original report indicated, the same dosages being used. It is noted in addition that the above reversion progressed quite slowly after the bacitracin was given in addition to the other two drugs, which is in

contrast to the usual reversion with streptomycin alone. Whether this slowing should be attributed to the aluminum pectinate or to the bacitracin cannot be determined from the current experiment; it would be interesting to start the bacitracin just after the coliform counts reached their lowest level, in an attempt to prevent such reversion. One cannot say whether the continually maintained low counts of anaerobic spore-formers should be attributed to the streptomycin or the bacitracin, but on the basis of previous work, the bacitracin seems more likely. The reduction in the number of anaerobes, though not as marked as in the case of other organisms and not maintained for any significant time, has not been seen with the use of any other drug or combination of drugs. Just what group of organisms was involved in that reduction cannot be stated with certainty, but it is quite possible that it is *Bacteroides*, a group of Gram-negative anaerobic rods about which little is known but which is thought by many to represent the greatest single component of the normal human intestinal flora. It is interesting to note that this reduction occurred only in the group receiving streptomycin first. It is possible that a more profound and consistently maintained reduction in numbers of this group might be obtained by adjusting the dosage of the drugs used or by changing the dosage schedule, such as administering the bacitracin earlier. Whether such a reduction would be of benefit from the standpoint of pre-operative preparation of the bowel or in nutritional studies is unknown.

The two subjects who started on bacitracin and then had streptomycin plus aluminum pectinate added showed no better response than patients maintained on bacitracin alone; this type of combination of the drugs appears to have no advantage. The rise in coliform counts, seen in these subjects after bacitracin was started, might be explained by the removal of the competition for nutrients offered by *Clostridia* and *S. fecalis*, by the removal of antibiotic products elaborated by these organisms, or by bacitracin serving as a growth factor for coliforms.

Summary

"Circulin" offers no promise as an intestinal antiseptic in its own right or in combination with sulfasuxidine.

The combination of streptomycin (with or possibly without aluminum pectinate) and bacitracin for intestinal antisepsis offers possibilities of results better than those obtained with either antibiotic alone when the patients are started on streptomycin first, and this combination warrants further investigation. The combination of streptomycin and bacitracin started together should also be investigated. The combination of bacitracin and streptomycin with glucoronolactone should be studied.

The addition of aluminum pectinate to streptomycin in order to prevent reversion of intestinal bacterial counts probably is not so effective as hoped.

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PRECAUTIONS IN THE USE OF PHOSPHATE BUFFERS WHEN STUDYING LIVING TISSUES *IN VITRO**

IRVING GALINSKY

The use of buffer solutions when making metabolic studies is almost universal. Tissues to be studied are generally placed in buffer solutions of the desired pH and then some metabolic function is determined. One of the common series used, in one form or another, is the phosphate buffer, of which the five better known are:

(1) Krebs-Ringer phosphate, (2) Na_2HPO_4—citric acid, (3) Na_2HPO_4—KH_2PO_4, (4) KH_2PO_4—NaOH, (5) Na_2HPO_4—NaH_2PO_4.

Since the inorganic phosphates (Na_2HPO_4, NaH_2PO_4, K_2HPO_4) have been shown by me to inhibit mitosis in concentrations as low as 0.025M (0.355%), further studies of living tissues immersed in these substances should be viewed critically with regard to possible untoward effect.

Masuoka and Saunders and also White and Salter have shown that muscle tissue in a carbonate buffer will continue to respond to stimuli over long periods of time without much decrease in force but in a phosphate buffer a rapid decrease in mechanical activity occurs. Concentrations as low as 0.00105 M phosphate buffer have been used, but the highest concentration, 0.00263 M was observed to produce the most rapid fatigue.

In examining papers on tissue activity published in 1950, 18 were noted in which phosphate buffers were used when studying some tissue function *in vitro*. In these the authors of 7 utilized Krebs-Ringer phosphate; 5 specified other phosphate buffers and concentrations; and 6 did not state which phosphate buffer was used. Two of the five fully reported buffers contained enough phosphate in the final mixture to inhibit root mitosis.

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In order to demonstrate what percent phosphate is present at various pH levels of the different phosphate buffers, the accompanying table has been prepared.

Buffer	pH	Amount of Phosphate Salt	Final Volume	Percent of Phosphate salt
Krebs-Ringer Phosphate	7.4	12.0 cc.–0.1M Na_2HPO_4	121 cc.	0.14
Na_2HPO_4—citric acid	3	4.11 cc.–0.2M Na_2HPO_4	20 cc.	0.58
	4	7.71 cc.–0.2M Na_2HPO_4	20 cc.	0.71
	5	10.30 cc.–0.2M Na_2HPO_4	20 cc.	1.46
	6	12.63 cc.–0.2M Na_2HPO_4	20 cc.	1.79
	7	16.47 cc.–0.2M Na_2HPO_4	20 cc.	2.34
	8	19.45 cc.–0.2M Na_2HPO_4	20 cc.	2.76
Na_2HPO_4–KH_2PO_4	6	12.0 cc.–M/15 Na_2HPO_4	100 cc.	0.26
	7	61.1 cc.–M/15 Na_2HPO_4	100 cc.	1.30
	8	94.5 cc.–M/15 Na_2HPO_4	100 cc.	2.01
	6	88.0 cc.–M/15 KH_2PO_4	100 cc.	1.80
	7	38.9 cc.–M/15 KH_2PO_4	100 cc.	0.81
	8	5.5 cc.–M/15 KH_2PO_4	100 cc.	0.11
KH_2PO_4–NaOH	6	50 cc. 0.2M KH_2PO_4	200 cc.	0.68
	7	50 cc. 0.2M KH_2PO_4	200 cc.	0.68
	8	50 cc. 0.2M KH_2PO_4	200 cc.	0.68
Na_2HPO_4–NaH_2PO_4	6	10 cc. 0.1M NaH_2PO_4	12.5 cc.	0.96
	7	10 cc. 0.1M NaH_2PO_4	39.0 cc.	0.31
	7.4	10 cc. 0.1M NaH_2PO_4	103.0 cc.	0.12
	6	2.5 cc. 0.1M Na_2HPO_4	12.5 cc.	0.28
	7	29.0 cc. 0.1M Na_2HPO_4	39.0 cc.	1.06
	7.4	93.0 cc. 0.1M Na_2HPO_4	103.0 cc.	1.28

The table shows that the phosphate concentration at most pH levels is higher than the 0.355% found to be effective in disturbing mitosis. The 0.355% concentration, while approaching the lower limit of effectiveness, may not be that limit itself. With this in mind it can be seen that within the more commonly used pH range of 6, 7, and 8, the phosphate concentrations vary from one to nine or more times the concentrations necessary to disturb mitosis in the roots of such plants as *Allium*, *Vicia* or *Tradescantia*. While non-mitotic tissues will survive higher doses of phosphate than will mitotic tissues, metabolic disturbances must occur since the tissues eventually die. An increasing fibrous appearance of the nuclei and also a change in cytoplasmic texture can be seen after a few hours. The longer the tissue remains in the buffer, the greater will be the changes which occur.

Of the phosphate buffers listed, the Krebs-Ringer phosphate appears to be the best, containing the lowest phosphate concentration. However, what is needed is a comparative study,

testing a specific metabolic function of a specific tissue utilizing the different types of buffers (barbital, phosphate, bicarbonate) at the same pH.

Unless all workers realize the importance of reporting exact types and concentrations of buffers used by them, it will prove a difficult task to duplicate or properly evaluate their experiments.

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A CRITICAL EVALUATION OF ANTI-RETICULAR CYTOTOXIC SERUM (ACS)

THOMAS S. GARDNER AND DAVID M. SPEAKER*

On June 23, 1941, the work of A. A. Bogomolets and his associates on a new therapeutic material—anti-reticular cytotoxic serum (ACS) was revealed to the Western World by a cable from Moscow. As news of ACS gradually spread, misconceptions regarding its purposes and proper application followed rapidly as the scant scientific data published in Russian journals was received in this country.

Early references to ACS emphasized its role as a stimulator of the reticulo-endothelial system (RES). These papers described its value in promoting wound healing, fracture repair and in various trauma of combat origin. Some authors expressed the hope that ACS would prove an effective agent in the treatment of premature aging and, perhaps, even a possible means of extending the life span. *The American Review of Soviet Medicine*, in its December, 1943, issue, published English translations of three articles on ACS and these served as the primary sources for most later investigators in the field in the United States. Bogomolets (25) discussed the general therapeutic action of ACS in terms of his own theory of the physiological significance of the reticulo-endothelial system. Marchuk (100) described a method of serum preparation and preservation and Linberg (92) discussed its clinical application in frostbite and battle injuries.

The first announcement of ACS in the United States created a flurry of newspaper publicity and subsequent popular articles in various periodicals. Many of the writers described ACS as a kind of "elixir" which would cure all of man's physical ailments and bring the blessings of longevity to all. As a result of this unfortunate situation some groups engaged in objective investigation of the preparation were importuned from all sides for quick cures for every ill imaginable. As time passed and no therapeutic miracles came forth, public

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opinion gradually changed, and this change was accelerated, in part, by the injudicious publication of some hasty and some ill designed experimental work which led many to the conclusion that ACS was either a failure or a hoax. This impression pervaded medical and other scientific circles to such an extent that ACS was generally considered to have been disproven as a valid therapeutic agent. During this period, however, a few groups remained uninfluenced by this rapid fluctuation of opinion and continued to carry through fundamental experimental programs with ACS employing previously developed techniques for work with other anti-organ sera.

Part of the source of the confusion and uncertainties which have attended the ACS program in this country can be traced to data offered by Bogomolets and his collaborators which is sometimes conflicting and frequently inadequate. The Russian investigators had not established optimum dosage levels. For example, Bogomolets (25) repeatedly emphasized the necessity of using very small serum doses—0.03 to 0.10 ml. of undiluted serum to be diluted ten times with normal saline solution, and with a minimum complement fixation titer of at least 1:100. Linberg (92) suggested 0.5–0.75 ml. of undiluted serum, while Marchuk (100) recommended 0.1–0.25 ml. of undiluted serum. In each case, the lowest dose was to be administered first, followed by two additional doses terminating with the highest recommended. This was to constitute a series of ACS treatments. This uncertainty regarding dosage, already considerable through this disagreement, was further intensified by a complete failure on the part of any of these authors to relate the dose he favored to the weight of the patient under treatment or animal under observation. Unfortunately, many investigators in the United States adopted the dosage level proposed by Marchuk (100)—possibly because it was the intermediate of the three. Subsequent work in this country has shown that even Bogomolets' dosage levels were too high. In some cases the dosage question has caused difficulties in other ways. For example, Marchuk (100) specifically stated that the serum prepared according to his technique was to be diluted 1:10 with sterile physiological saline solution and stored under refrigeration for two days. The serum decays

in potency much more rapidly after dilution than when fresh and, under the procedure above, the titer is apparently reduced. This point has, however, been on occasion ignored in this country with the result that sera of too strong concentration have been used due to *immediate* application of the diluted preparation. On the other hand, some investigators here have made the opposite mistake of using diluted serum which had been permitted to stand for several days, resulting in a titer well below any effective level. Considerable work was done employing ACS prepared in this manner with, of course, negative results.

Another source of confusion has arisen from the fact that many investigators renamed ACS in describing their work. For example, a complete series of fundamental studies on anti-reticular cytotoxic serum, using tissue culture and animal investigations, which verified many Russian claims has been published. In this series, the preparation under study is called "reticulo-endothelial immune serum (REIS)." Verification of the wound healing effects of ACS was published in which the agent is called "anti-splenic tissue serum (ASTS)." A possible explanation of the latter name is that it has been established (94, 95, 96, 97) that spleen can be used alone as an antigen instead of spleen and bone marrow to prepare ACS.

In the past, a few short, technical reviews of ACS literature have appeared. Provenzale (134) and Morhardt (113) in Europe discuss the subject briefly. In this country Pomerat (131), Strauss (146, 149), Ilfeld (72) and Norgore (118) have presented qualitative review papers. These summaries were uncritical of experimental techniques, however, and did not discuss correlation of their work with that of other investigators. To fill the need for a more complete survey of the field, and in the hope of a more complete clarification of the current status of ACS and its historical development, it was considered desirable to prepare a comprehensive and critical analysis of all work in the field in this country of which the authors have knowledge with sufficient discussion of Russian work to serve as a background. In this paper an effort is made to correlate procedures, dosages, techniques of serum preparation and syndromes under study with the aim

of a more correct evaluation of ACS. Such an approach is felt necessary if ACS is to be considered as a therapeutic agent.

In this paper, all investigations under study are analyzed wherever relevant, on a basis of dosage level in relation to the weight of animal or patient. Where this data is not included in the report, estimates are made based upon known average weights of experimental animals and patients. All dosages are reduced to a dose-weight factor expressed in Bogomolets' Units (95) per kilogram. The Bogomolets' Unit was originally proposed by Malisoff (94, 95) and is defined as follows: Titer of serum $\times$ ml. $=$ Bogomolets' Units (B. U.). For example, 0.1 ml. of a serum having a complement titer of 1:340 would have a potency of 34 BU.

Russian Investigations of Anti-Reticular Cytotoxic Serum

Metchnikoff originally proposed the use of tissue anti-sera as medical therapeutic agents (109), but at that time no accurate techniques existed whereby serum potency could accurately be determined experimentally. Practically all early work done at that time involved too high dosages and cytotoxic effects were predominant. Bogomolets, a pupil of Metchnikoff (65), applied the technique of complement fixation to the problem of dosage control and used small, stimulating doses based upon earlier proposals of Metchnikoff and others. Because of this, Bogomolets is generally given credit for having been the first to place ACS on a sound basis for investigation as a therapeutic agent. Fundamental to Bogomolets' conception of ACS and its physiological action was the emphasis he placed upon the reticulo-endothelial system (RES) as a functional, dynamic entity both in health and disease (16, 18, 20, 24, 25, 26, 27, 152). Bogomolets was impressed with the importance of the healthy condition of the RES in very old people as shown by their prolonged resistance to disease and degenerative tissue changes. Clinical work with ACS, as well as experimental studies of the reaction of the RES to ACS by Tartinov (152), Kavetskey (80), Kavetsky and Pasternak (78), and Goldfarb's (62) work in tissue culture, partially verified Bogomolets' conception of the importance of the RES

in health and disease as well as the susceptibility of the RES to stimulation by ACS.

Bogomolets and his associates developed techniques for the preparation of ACS. In particular, the methods developed by Marchuk (99, 100, 102) have been used as models by American investigators. Subsequently, improvements have been made which are described later in this paper. Essentially, ACS develops as a product of anti-body response to spleen and bone marrow antigens. The antigenic material was injected subcutaneously or intravenously into another species of animal which subsequently developed anti-bodies against the antigen. The serum thus obtained was standardized and used under controlled conditions. This method is conventional for the production of anti-sera, but the modifications and improvements introduced by Bogomolets and his associates placed it on a reliable basis for the first time. Fundamental researches progressed concurrently with clinical applications. Timofeevsky and Benevolenska (156) investigated the stimulating effects of ACS on human tissues. Medvedeva (107) found effects on the metamorphosis of some amphibians. The relationship of hormones to ACS was investigated by Kavetsky (76), while Tartinov and Zilberman (151) explored its hemotological action, finding a rise in monocytes. Medvedeva (104, 105) found that favorable changes in the composition of blood, protein and nitrogen compositions of tissues took place with ACS. At the same time, clinical work with ACS showed that it was contraindicated in endocarditis and myocarditis (25, 28), especially if administered intravenously (25).

O. A. Bogomolets (30, 31, 32, 33) reported that ACS increased the healing rate of fractures, improved the formation of bone callus and materially reduced the time required for recovery from bone injuries. This work was carried out on both experimental animals and clinical patients.

The rate of wound healing is a good indication of the general state of the physiological system, especially the RES. Kogan (87) found that ACS favorably influenced the rate of wound healing, while Linberg (92) investigated war wounds of various types as well as frostbite. Early wounds and wounds showing delayed healing were improved. Even apparently

hopeless cases were reported to have progressed remarkably with eventual complete recovery.

The level of activity of the RES appears to have an important influence upon the body's ability to combat both malignant and benign tumors, and it has been found that at least partial protection against malignant implants in experimental animals were obtained by the use of ACS. In inoperable cases of human cancer, ACS was found to reduce pain and appeared to prolong life beyond the expectancies reasonable under the circumstances (25). ACS has also been found beneficial as a general prophylactic treatment after surgical removal of cancer and/or irradiation. In such cases, the incidence of recurrence of malignancy appears to be reduced. Generally speaking, investigations of the therapeutic value of ACS in cancer, both clinical and experimental, have been extensive (17, 37, 38, 43, 45 51, 52, 53, 54, 55, 77, 141, 150). The concensus is that although ACS is definitely not a cure for cancer there is evidence that it tends to prevent relapses following surgery, reduces pain in inoperable cases and generally improves the state of well-being of the patient. Consequently, it could be regarded as an important adjunct to the standard therapeutic procedures commonly employed in malignancy.

Ulcerative conditions of the body frequently acquire the quality of permanence, suggesting a decreased local capacity for growth. In this connection, the stimulating effects of ACS are especially important, renewing the cell's ability to grow and thus heal the ulcerative condition. Beneficial therapeutic applications of ACS to skin ulcers have been reported by Mashkilleison (103), to peptic ulcer by Orlova (122), and to gastroduenal ulcers by Kuskiy, Kravenchenko, and Apollo (88), and to culcerative cicatrix by Berezov (12). Abscesses also respond favorably following cellular stimulation by ACS according to Mikhnev (112) and Varchamov (157).

Iosefova (73) reported that ACS could be employed in certain conditions of ocular tuberculosis, particularly in chronic torpid keratitis profunda. Partashnikov (123) claimed that the course of typhus was checked by ACS, and this was also reported by Strazhesko (92). Favorable claims have also been

made for the use of ACS in hypertension (19, 25) in which it was said that it produced a permanent reduction in blood pressure to normal values in most cases. ACS has been reported of value in septic angina as used alone (81), with vitamin supplements (2) and with sulfonamides (83). It has also been tried in myocardial infarction (48) and has been contraindicated in myocarditis (25).

Beyond the applications noted above, ACS has been employed therapeutically in a variety of pathological conditions. These include: allergies as reported by Spasokukotskiy (143), diseases of the eye—Dyban (47), ozena—Mihailovsky et al. (111), and various internal diseases—Tatevosov (153) and Kavetsky and Kavetsky (79).

Probably one of the most challenging problems of modern medicine is the development of an effective treatment for schizophrenia. Various claims have been made for the therapeutic value of ACS in several types of psychopathies, especially schizophrenia, by Protopopov (133), Zalkind and Chernobrovk (160), and Bogomolets and his collaborators (22, 23). Any avenue of attack on schizophrenia offering hope is worth investigating further to the fullest extent.

In the field of gynecological and obstetrical conditions, Serdyukov (139) reported amazingly good responses to ACS therapy. He treated 425 patients for inflammatory diseases of the genital tracts, menstrual disorders, fibrotic metropathies, subserous fibroyomas, endocrince and climacteric disturbances, hypogalactica, toxemias of pregnancy, precancerous lesions, and various complications such as gastric ulcers and stomatitis.

Kavetsky (82) investigated the use of ACS in treatment of diseases of the digestive system. His cases number over 200. ACS was used as an adjunct to dietotherapy and atropin. Positive results were reported in about 80% of the gastric and duodenal ulcers with complete recession in 22% of the cases. Chronic gastritis patients demonstrated complete absence of symptoms, after treatment, in about a third of the cases and appreciable improvement in half of them. Failures were experienced in about 17%. Chronic colitis patients experienced disappearance of diarrhea, decrease of pain and other symptoms, with improvement in general well-being. Inoperable

cases of cancer of the stomach and the esophagus obtained relief from pain, increases of body weight, and some general improvement. No cases of complete suppression were noted.

Varchamov (157) observed improvement in typhoid and pulmonary abscesses, while Volkov (159) reported favorable results in ACS treatment of experimental colibacillosis. Partashnikov (124) also obtained positive benefits in pulmonary abscesses, pneumonia and brucellosis. Early suppression of scarlet fever was reported by Bogomolets and Marchuk (15, 26). A therapeutic effect on malaria was described by Exemplarskaya (50).

A rather unusual application of ACS was reported in which the agent was employed to raise the titer of diphtheria antiserums (101).

A summary of early clinical observations has been made by Dinerman (46). A general discussion of the therapeutic effects of ACS by Bogomolets (21, 26) emphasizes a point which the present reviewers feel is of importance. That is, ACS is not to be regarded as a sole therapeutic agent for any disease or pathological condition, but rather as an adjunct to other forms of therapy. The proof or disproof of the benefit of ACS in various syndromes may be investigated through animal or clinical work occasionally on the basis of exclusive therapy in part. But insofar as general use is concerned, it should be always kept in mind that there are two factors involved in the treatment of disease. One of these constitutes the addition of extrinsic factors such as antibiotics, sulfa drugs, surgical intervention and other general therapeutic techniques. The other embraces the intrinsic reparative mechanisms of the body itself. In other words, the body must heal itself as well as be healed. That is wherein the value of ACS lies—not as a specific therapeutic agent in itself, but as a stimulator of the body's own front line defense and repair agency—the reticulo-endothelial system (RES).

The work of Russian investigators in exploring the possible applications of ACS to the problem of aging will be discussed in a later section of this review dealing with that general topic.

The Physiological Actions of Anti-Organ Sera

The concept of anti-organ sera probably originated with Bordet (35) who prepared a cytotoxic serum against erythrocytes, and was developed by Metchnikoff (109) with the production of an anti-spleen serum using emulsified rat spleen injected subcutaneously into guinea pigs. He showed that the anti-rat spleen serum recovered from the guinea pigs was cytotoxic to rat lymphocytes. He also prepared an anti-rabbit lymph node serum. From this early work two fundamental properties of anti-organ sera was observed: 1. Cytotoxic effects from large doses. 2. Specie specificity.

Cantacuzène (36) prepared anti-erythrocytic serum and confirmed that large doses were cytotoxic, but also discovered that small doses produced stimulating effects. His rabbit anti-erythrocytic serum, used at a level of 1/8 ml., produced an increased red cell count which remained high for over thirty days. This effect was verified with anti-leukocytic serum by Besredka (13). A rise of leukocytes was obtained by the use of small dosages, and thus the third fundamental property of anti-organ sera was established—stimulating effects from small doses.

Most of the investigations of anti-leukocytic sera and anti-lymphocytic sera have been on their cytotoxic effects, sometimes called "blocking" effects. The properties of such sera were investigated in tissue culture by Hueper and Russel (71), and *in vivo* by Funck (59), Chew et al. (39, 40), and Cruickshank (42). The destructive effects of large doses of these sera has been fully verified by these studies and are generally characterized by the death of the treated animal in a short period, always preceded by a fall in the lymphocyte or leukocyte count and accompanied by histological organ changes.

The application of tissue culture techniques to anti-organ sera investigations has enabled controlled studies of specific cellular response. This procedure eliminates the necessity for the large number of experimental animals usually needed to establish meaningful statistical values due to the variability of *in vivo* results. A few brief references to the application of tissue culture work to general anti-sera investigations will be

helpful as a background for the work as applied specifically to ACS.

Bedson (11) investigated the specific action of blood platelet anti-serum. Immune reactions in tissue cultures were demonstrated by Bloom (4), and Kimura (84, 85) investigated rabbit anti-whole embryo serum on cells *in vitro*. Fellinger (56) worked with anti-fibroblastic serum in tissue cultures. Spassky (144) showed that large quantities of cytotoxic serum inhibited growth and metabolic activities of tissue *in vitro*. An anti-kidney serum was prepared by Verne (158) who investigated its effects on tissue cultures. It is interesting to observe that the cytotoxic or blocking effects of these anti-sera dominated attention to the practical exclusion of their stimulating potentialities. An analysis of the blocking effects in terms of tissue culture studies was carried out by Kimura (85), for example. However, Lasfargues and Delaunay (89) investigated the general problem of the mechanism of action of anti-leukocytic sera on tissue cultures and they reported (90) that Bogomolets' ACS stimuated the growth of cells *in vitro* when very diluted quantities were used, and inhibited their growth in larger amounts.

A firm background of scientific investigation had been layed of anti-sera reactions *in vitro* before American investigators dealt with the problem of appraising the Russian claims for ACS and of attempting to duplicate and extend their work.

Probably one of the most complete and thorough investigations of anti-sera carried out in America was that of Henle et al. (69). In this brilliant study, the fundamental properties of anti-organ sera were verified and further developed. In fact, the properties of ACS could have been broadly predicted from Henle's work. In his investigations, several anti-organ sera were prepared from brain, liver, lung, kidney, spleen, testicle and pancreas as antigens. The preparation of the corresponding anti-sera was carried out in essentially the same manner as for Bogomolets' ACS. All anti-sera were titrated by complement fixation—this application of standard serological technique to anti-organ sera being, by the way, one of Bogomolets' greatest contributions to the development of ACS. All anti-sera exhibited at least some degree of organ

specificity except for pancreas anti-serum. With the exception of the brain anti-serum, all anti-bodies developed were thermolabile, being destroyed by heating to a temperature of 100° C. for a few minutes. The brain anti-serum, however, retained practically undiminished complement fixation titer under these circumstances. The brain anti-serum producing antigen was found present in the alcohol and ether extracts from brain tissue, while these extracts of other organs had no antigenic properties. This is in good agreement with the later work of Frieden, Pomerat, and Anigstein (57) who located ACS, the anti-serum prepared from bone marrow and spleen, in the globulin fraction of the blood. Henle (69) was unable to demonstrate isoimmunization.

It is interesting to note that Henle's investigations, generally speaking, would have the basis for a complete and parallel development of ACS in America without the benefit of data from Russian sources, given the knowledge that such a serum had therapeutic value.

Pomerat and Anigstein initiated and conducted a long term investigation on ACS with both *in vitro* and *in vivo* phases. Perhaps unfortunately, they changed the name anti-reticular cytotoxic serum (ACS) in their papers to reticulo-endothelial immune serum (REIS). Because of this, it is believed that many investigators may have been unaware of their work as part of the general ACS investigation in this country.

Their first reports (125, 126) described tissue culture experiments in which ACS exhibited stimulating effects in very dilute solutions and cytotoxic effects in higher concentrations. This was the first direct verification of the properties of ACS prepared from spleen and bone marrow to appear in America. Pomerat and Anigstein (127) reported later that chick ACS (complement fixation titer 1:1200) had a stimulating effect at 1/400 dilution, and cytotoxic effects at 1/4 dilution on chick heart fragments. Chick, rat, and guinea pig ACS (C.T., 1:1200) were also prepared (128). In introducing spleen anti-serum to spleen tissue cultures it was found that dilutions of the 1/20 produced marked blocking effects consisting of inhibition of outgrowth and clumping of migrating cells. The authors pointed out that the result of the direct use of spleen and

bone marrow antigens was not the same as that of the anti-sera prepared from them.

Pomerat reported that *rat* ACS had no inhibiting or stimulating effects on *chick heart* fragments *in vitro*. This verified Henle's work (69). However, as has been seen above, both effects were obtained with *chick* ACS. It was observed, interestingly, that ACS might have a greater stimulating effect on tissue cultures previously treated with 25% embryonic extract. This synergism between embryonic extracts, generally regarded as growth stimulators, and ACS is of interest from the fundamental standpoint.

Pomerat (130) found that rat sarcoma 319 could be inhibited *in vitro* by rat ACS, but not by chick ACS. A complement fixation titer of 1:1600 inhibited the sarcoma at a dilution of 1/4, but not of 1/16. Further effects of strong dosages were demonstrated by Anigstein and Pomerat (3) in the bartonella carrier rat (4) by use of 0.5 to 1.0 ml. of rat ACS. This produced severe and acute bartonellosis with concomitant damage to the RES. The complement fixation titer used was 1:1600. Further evidences of the cytotoxic action of anti-sera was described by Pomerat et al. (132) in reference to work with blood anti-serum. Single injections in the bartonella carrier rat of anti-blood serum developed anemias of a degenerative type which were different from those produced by blocking doses of rat ACS.

Ewing and Emerson (49) found no beneficial effects on Trypanosoma equiperdium infections in mice using ACS. Details do not appear in the report. Thomas, Ewing and Emerson (154) investigated the potentialities of spleen cytology as the basis for a bioassay method for ACS. Their findings indicate irregular blood changes except for a "dramatic regular change in the distribution of small lymphocytes . . . in the spleen with migration from the periphery of the Malpighian corpuscles into the cords of Billroth."

The precise mechanism of ACS stimulation and inhibition of the RES is still an unsolved problem. In an effort to gain some insight into the basic nature of ACS action, some studies of its effects on tissue respiration have been made. Cohen, Runjavac and Strauss (41) reported that ACS prepared from

guinea pig antigen, and also ACS from rabbit spleen and bone marrow, had no effect on the respiration of guinea pig or rabbit bone marrow, respectively. Both undiluted ACS (0.1 ml.) and half dilutions were used with 2.0 ml. of bone marrow suspension. Nowinski (120) checked these observations with rat ACS on rat spleen slices and found no inhibitory effects on oxygen consumption. Concentrations of 1/4 were used in order to ensure inhibiting quantity of the agent if any such effect were to appear. As these two separate studies agree very closely, it can be at least tentatively concluded that the blocking effects of ACS in tissue cultures are not due to inhibition of cellular oxidative processes.

MacDonald (93) measured the dehydrogenase activity of rat spleen in the presence of rat ACS. He found that rat ACS from dilutions of 1/10,000 up to undiluted serum (C.T., 1:20 to 1:1500) had neither a stimulatory nor an inhibitory effect upon the dehydrogenase activity of rat spleen.

In contrast to the lack of effect of ACS on tissue oxidative rates, Nowinski (121) found that while chick anti-brain serum with low complement titers of 1:30 and 1:50 were without significant effects on the rate of oxygen uptake of chick brain homogenates, stronger chick brain anti-serum (C.T., 1:500) had a quite perceptible, though not high inhibitory effect. (ACS having a C. T. of less than 1:100 is considered too inactive for effective use. By analogy, the anti-brain sera of Nowinski of low titers, i.e., 1:30, 1:50, were probably too low in concentration to be effective and only his results with the 1:500 titer anti-brain serum should be compared with those with ACS).

A distinct difference in effect on cellular oxidation rates is therefore found between anti-organ sera prepared from brain and from spleen-bone marrow (ACS). Pending further evidence, if one wishes to speculate, one may suppose that possibly anti-organ sera will exhibit different effects upon different enzyme systems. Grunwaldt (64) investigated anti-brain serum using tissue culture methods and found that two different antigens could be demonstrated in the chicken brain. They could be separated by their differences of solubility in alcohol and Grunwaldt showed that these two fractions had

different effects on embryo chick cords of different ages. Insofar as ACS is concerned, it may be the product of a homogeneous antigen, although doubt has been expressed upon this point by Kling (86). One argument in support of the homogeneity of the antigen is that ACS prepared from spleen alone or from spleen and bone marrow exhibit identical properties, both stimulating and cytotoxic. Certainly, it is fortunate that ACS can be prepared from spleen as a sole antigen because of its greater availability as compared with bone marrow.

Pomerat has suggested (4) and has employed a method of ACS assay by tissue culture techniques in which the serum action on the growth of the explant is evaluated. This method was sometimes used when anticomplementary action of the antigen prevented titration. The use of tissue culture techniques as an analytical tool has not been developed, although Ivers and Pomerat (74) have shown that ascitic fluid from patients containing malignant elements inhibits fibroblastic proliferation in tissue cultures and they suggest this as the basis of a possible diagnostic technique.

There is now little doubt that the fundamental tissue culture investigations of ACS in this country have in the main verified the basic contentions of Bogomolets insofar as the stimulating, inhibiting and general anti-organ properties of the agent are concerned. The final test for any therapeutic preparation is, however, *in vivo* data. These are discussed in the next section of this review.

Research and Clinical Studies of ACS

Animal and clinical investigation of ACS have been pursued in this country by both institutions and private physicians. The resulting literature is appreciable, embracing a considerable range of therapeutic applications as well as preparative techniques. In the following section of this review an attempt is made to analyze this work with special reference to dosages employed and to correlate it, wherever possible, with the claims of Russian investigators.

Improvements in the preparation and titration of ACS in this country have stemmed largely from the work of Strauss and

his co-workers (147), and of Maltz and his group under Malisoff's direction (95, 96). Strauss cross-checked his preparations with Bogomolets' sera for titer comparison and made studies of the effect of the age of the antigen on serum titer. Maltz's group worked on the problem of antigen preservation, demonstrating that the antigen could be kept for considerable periods at —20°. Strauss found that undiluted ACS was stable at 4° C. for one month without loss of titer, but that a loss was observed after two month. Lyophilization increased the 4° safe storage period to several months. In the case of one ampoule it was found that the titer at the end of a year had dropped from 1:1280 to 1:160. The latter titer is still usable, but it is apparent that serum in its present stage of development has a limited shelf life.

A very careful study of the preparation of ACS from spleen and bone marrow, and its subsequent titration, was carried out by Movitz, Howell, Saphir, and Strauss (114). Anigstein, Whitney, Pomerat and Orr (5) reported that small amounts of antigen, inoculated at successive intervals, were more effective, as measured in terms of subsequent antibody titer than attempted rapid immunization with single massive doses of spleen and bone marrow. An anamnestic response could be obtained through preliminary sensitization with the antigen followed after 30–40 days with another inoculation. This technique was found to yield sera of high potency.

An important advance was the demonstration that ACS could be prepared from spleen as an antigen alone without bone marrow added. Miale (110) reported that dog spleen antigen gave a serum of high titer (1:500) which in dosages of 0.004 ml/kg in dogs resulted in a significant increase in the circulating leukovyte count—specifically in the lymphocytes. The effect lasted for about 20 days. However, blocking doses of 0.54 ml/kg depressed the count. The lower dosage (which equates to 2 BU/kg) is on the border line between stimulating and blocking levels, while the higher dosage (250 BU/kg) is a massive blocking dose. An extensive investigation by Malisoff with Maltz, Spain, and Molomut (94, 95, 96) clearly confirmed that ACS prepared from spleen alone was equivalent to that prepared from spleen and bone marrow. This conclusion was again verified by Heiman and Meisel (66, 67, 68). Malisoff

and his co-workers (94, 95, 96) studied the effects of intraperitoneal vs. intravenous administration of the antigen. The intravenous route increased the number of deaths of animals from analphylactice shock and this is one reason why intraperitoneal inoculation is generally considered the favored technique.

One of the most important problems involved in the possible use of ACS as a therapeutic agent is that of optimum dosage for maximum stimulation of the RES. Bogomolets (25) and Marchuk (100) stated that a titer of 1:100 represented the minimum useful serum strength. The most powerful human ACS prepared by Marchuk (100) was 1:240. However, Bogomolets (25) and Marchuk (100) differed on the preferred level of stimulating dose for human use. Bogomolets (25, 29) recommended three doses—0.03 ml., 0.05 ml., and 0.10 ml.—administered over a ten day period constituting what he termed a "series." This represents a total of 0.18 ml. and, at a complement titer of 1:100, equals 18 BU for an adult. Assuming an average adult weight of 70 kg, this is equivalent to 0.26 BU/kg. For Marchuk's highest titer of 1:240, the equivalent dose is 0.60 BU/kg. However, Marchuk (100) recommended "0.1–0.3 ml" and this has been interpreted by several investigators in this country to mean a series of 0.05, 0.10, and 0.15 ml. or 0.30 ml. total. On the basis of the minimum acceptable complement titer this is 0.43 BU/kg and at the maximum reported titer it is 1.0 BU/kg. However, no data was published to indicate whether a careful screening of various dosage levels had been made. Such an investigation may be assumed but, if it was ever carried out, we are unaware of the results. That the above dosage levels are not optimum, being apparently too high, is suggested in reports (94) from Russian physicians during the war who stated that on the battlefield whenever their ACS supply ran low it was extended by dilution with normal saline and, much to their surprise, produced superior results.

The three following investigations to be cited are relevant to the problem of optimum ACS dosage, though they are discussed additionally elsewhere in other connections. They deal with bone healing in rabbits (148) and fracture healing in clinical

patients (155); wound healing in mice (94, 95, 96); and inhibition of the Brown-Pearce carcinoma in the rabbit (115). All of these agree in that the dose levels recommended by Bogomolets and collaborators is too high. Strauss and his co-workers (148) found that 0.15 BU/kg was a stimulating dose as administered to rabbits with artificially produced fractures and that this level gave excellent repair rates. On the other hand, 13 BU/kg was a blocking dose and depressed the bone healing rate. In a quite extensive investigation covering over a thousand skin wounds in mice a complete screening program was conducted under the direction of Malisoff (94, 95) in conjunction with Maltz and his co-workers (96). They found that 0.10 BU/kg was the best stimulating dose for the acceleration of wound healing. Movitz and his associates (115) in a careful investigation of the Brown-Pearce carcinoma in rabbits verified the dosage level recommended by Strauss (148) and reported that 0.08–0.11 BU/kg represented a range of stimulating levels as administered in a series of two or three equal doses about 9 days apart. Further verification of the small dose schedule over a 42 day treatment period was reported by Thompson et al. (155) in connection with a series of over 7,000 human fracture cases in which Strauss' ACS was employed.

On the basis of the best exprimental evidence available thus far, both with animals and clinical patients, it would appear that the best stimulating dose level is 0.1–0.15 BU/kg used in a single injection to be repeated, if necessary, about every three weeks. Doses above this level may generally be expected to produce poorer results and at about 1.0 BU/kg, some blocking effects may occur. However, there is some evidence that stimulating effects may be elicited in some cases up to 2.0 BU/kg (110). In all instances definited blocking and depression of RES functions are apparent at a level of 13 BU/kg (110). The following analyses of results obtained in this country will be referred to these levels. It will be seen that for the vast majority of the cases published the size of the dose employed will be of value in interpreting the experimental data.

a. Wound Healing.

Nickerson, Burns and Cooper (117) reported that massive doses of ACS—at blocking levels—did not increase the healing

rate of experimentally produced wounds in rats. Animals were given a series of three injections at three day intervals. The dosage levels were 0.25, 5.0, 40 and 600 BU/kg for each series. As the stimulation period in rats, mice, dogs, guinea pigs and man are about three weeks, these series may be evaluated in terms of equivalent total doses. These are 0.75, 15, 120 and 1800 BU/kg and these are far above the levels at which any wound healing effects would be anticipated. It is, in fact, rather surprising that *decelleration* of wound healing was not reported.

The comprehensive investigation under Malisoff's direction with Maltz, Spain and Molomut (94, 95, 96) on wound healing in mice is significant for two reasons. It verified the basic Russian claim that ACS did stimulate wound healing and it also established the optimum stimulation dosage level to be 0.10 BU/kg. According to these authors, a single injection was found superior to a series of doses. In one experiment, after eight days control mice were 40% healed, while mice treated with 0.10 BU/kg of ACS were 72% healed. These figures are statistical averages in over a thousand standard wounds. Also, mice treated with anti-mouse ACS, previously freed of anti-red cell factors by absorption on red blood cells, exhibited 79% wound healing as compared with controls. Pretreatment with ACS, prior to wounding, had no effect on the healing rate.

Maltz, Spain and Molomut (97) reported that anti-guinea pig ACS accelerated the healing of large wounds of guinea pigs when used in a series starting with 0.8 BU/kg. In this series the dose was repeated every three days, tripled in concentration, with a fifth dose on the 14th day. At the end of the 8th day, controls were 17% healed and treated 32%; on the 14th day controls were 65% healed and treated 84%. The healing rate dropped off markedly after the 14th day. It is quite probable that a single injection of 0.10 BU/kg would have given better results as the total series above involves a dosage level within the blocking range. Stimulating effects may have developed from the initial injection and carried over in spite of the high later doses. For comparison purposes, it is interesting to note that Maltz (98) prepared non-specie specific anti-sera from mouse and human granulation tissue that accelerated wound healing in doses of 0.5 and 0.15 units/kg, and produce blocking

actions in mice using the mouse anti-granulation tissue serum at a level of 12.5 units/kg. The human anti-granulation tissue sera, though exhibiting stimulating properties, did not produce blocking on the same level in mice as the mouse anti-granulation tissue serum.

b. Fracture healing.

One of the more important claims for ACS by the Bogomolets (25, 28, 30, 31, 32, 33) was its ability to accelerate the healing rate of bone fractures. A broad investigation of this possible therapeutic application of ACS was conducted by Strauss and his co-workers (148, 155). Strauss (148) reported that the healing of experimental fractures in rabbits had been accelerated by single injections of ACS at dosage levels of 0.15 BU/kg. Roentgenographic examinations of the bones indicated a greater degree of callus formation and calcification in the ACS treated group. Blocking doses of 12.8 BU/kg depressed the healing rate and callus formation. The abilities of the healed, or partly healed bones to resist fracture were measured as one criterion of degree of healing. It was found that rabbits treated with stimulating doses of ACS had developed in their legs which were permitted to heal for a fixed period following fracture, about 8.5 times the breaking strength of those rabbits which had been given blocking doses. Also, the rabbits treated with stimulating doses of ACS exhibited about twice the breaking strength of the bones as compared to control animals injected with normal goat serum.

Strauss and his collaborators (155) extended these studies to include clinical cases of all ages. Over 7,000 patients totaling over 25,000 injections of ACS intramuscularly or subcutaneously were used in this series. No significant side reactions were noted. Some patients proved sensitive to rabbit processed ACS and these were treated with goat processed ACS instead. Small stimulating doses were given over a period of 42 days. The first injections corresponded to 0.046–0.368 BU/kg, depending on the titers of the sera used which varied from 1:160 to 1:1280. At the end of the third day the injection was increased to 0.05 ml of serum and 0.10 ml for the third injection. The dose was gradually raised to 0.25 ml for the sixth injection which was reached at the end of 18 days and then maintained at this

amount for six more injections. It is possible that better results might have been obtained at a level of 0.1–0.15 BU/kg for a single injection, repeated every three weeks if necessary. Strauss reported high rates of callus formation and healing. An evaluation of roentgenographic bone studies and of the general clinical progress of the patients led to the following estimates: ACS treated—good, 62%; average, 38%; poor, 0%. Controls—good, 31%; average, 56%; and poor, 13%. The use of ACS in cases of delayed healing of old fractures gave good results as compared with the previous clinical histories of these patients. It is recommended that this paper be studied for complete details (155).

A further verification of the value of ACS in fracture healing appeared recently (138). Pomerat and his collaborators employed ACS in the treatment of victims of the Texas City disaster in 1947. They reported stimulation of healing using the early Russian dose schedule of 0.05, 0.10, and 0.15 ml. of serum. Assuming a minimum titer of 1:100, this is equivalent to about 0.4 BU/kg. Generally, the healing acceleration they obtained was somewhat less than that reported by Strauss (155). In neither case was the optimum dosage level used.

c. Rickettsial Infection.

Bogomolets (25) claimed benefits from the use of ACS in rickettsial infections. Due to the subsequent development of aureomycin, terramycin, and chloroamphenicol in this country, diseases of the rickettsial origin are no longer regarded as seriously as in former years. However they are of interest as one basis for the evaluation of ACS. Anigstein and his group (6, 7, 8) conducted investigations on guinea pigs in this field using ACS dosage levels of about 3 BU/kg as estimated from the given data. These were reported to give protection from typhus and spotted fever when the rickettsia were injected intradermally two hours after and at the same place as the ACS. The control animals developed the diseases.

Anigstein et al. (8) also employed ACS diluted with testicular extract containing the Duran-Reynals "spreading factor." The guinea pigs used in these experiments were injected with rickettsia at sites two inches away from the points at which the ACS was injected, and also at the site of the ACS injection.

Two control series included animals injected with rickettsia, and with normal rabbit sera along with the rickettsia for one series, and the second series had the rickettsia injection and guinea pig ACS diluted with normal saline. Both control series developed typhus or spotted fever respectively. However, the spreading factor caused the ACS to protect the animals from the rickettsia even when injected at a different site from that at which the organisms were introduced. Also, the protected afebrile animals were immune against reinfection when re-inoculated with rickettsia one month subsequent to the original inoculation. The use of hyaluronidase as a spreading factor together with ACS may thus be indicated for certain therapeutic uses. Further investigations will be required to evaluate this combination.

d. Cancer.

Bogomolets and his collaborators claimed that stimulating doses of ACS decreased the incidence of relapse following treatment of malignancy by surgery or radiation. They made the additional claim that inoperable cases of cancer had reduced pain with ACS. The second claim has been checked and verified by Goldblatt (44, 61) and by Malisoff (94). It should be emphasized that Bogomolets and his co-workers never maintained that ACS was a *cure* for cancer; they stated only that it was useful as an adjunct to standard therapeutic procedures.

An early investigation of the use of ACS in Hodgkins' disease was conducted under the direction of Malisoff (94) in conjunction with Skapier (140). They reported that ACS from Malisoff's and also from Goldblatt's laboratory used at the upper limit of the stimulating dosage range (0.5–1.0 BU/kg as estimated from data given) in a series of three injections had a distinct effect upon the course of the disease. General clinical improvements were noted as well as favorable hematological changes. However, if a lower dose had been employed (95), it is possible that better results may have been obtained.

In contrast to the positive results obtained by Malisoff and Skapier (95, 140), Abernethy and his co-workers (1) were unsuccessful. They employed a serum preparation from Goldblatt's laboratory at dosage levels of 12 BU/kg in four doses distributed over eight days of increasing amounts and at dosage

levels of 3.6 BU/kg in three graded doses over six days (estimated on the basis of an assumed minimum effective titer of 1:100). Such dosage levels are definitely in the blocking range and it is not surprising that these investigators reported negative results. It is amazing, however, that they employed doses of these magnitudes in view of the fact that the lower stimulating range was known to them and even discussed in their paper. Their report serves as an excellent confirmation of the blocking effects of high dosage levels of ACS as originally described by Bogomolets and his co-workers.

Following a very well designed investigation which is comparable to Strauss' fracture studies (146, 147, 148, 155), Movitz, Saphir and A. A. Strauss (115) of the Michael Reese Hospital in Chicago reported on the effects of ACS on the Brown-Pearce carcinoma of the rabbit. Having first established that large doses were without therapeutic effect, they then screened for optimum levels and established the stimulating dosage range at about the same as had been used by Strauss in rabbit bone fracture work (147). These investigators found that a total dose of 0.08–0.11 BU/kg in equal amounts at about nine day intervals materially suppressed the Brown-Pearce carcinoma in the rabbit. Movitz and his group also determined that either a too small dose, i.e., ineffective, or a too large dose, i.e., blocking permitted the malignancy to grow unchecked. This paper is recommended for the details of the design of the experiments as well as for the data contained.

Heiman and Meisel (66, 67, 68) reported on a series of investigations of the effect of ACS on malignancy in rats and mice. Unfortunately their dosage levels were in the blocking range. Their ACS was prepared from spleen and varied in complement titer from 1:160 to 1:640. In their first investigation (66), doses of 3.8–15.3 BU/kg and of ten times these levels did not decrease the development of adenofibromas, sarcoma 39, and R2426 rat carcinoma. (Dosage levels above were computed on the assumption of the average weights of rats as 250 gms.) Heiman and Meisel (67) found that mice injected with the highest blocking level doses described above developed mouse sarcoma 180 after tumor implantations and died. However, they also observed that massive blocking doses of ACS (68)

materially decreased the incidence of spontaneous mammary carcinoma in R3 mice. This, though, is most certainly not a demonstration of the stimulating action of ACS because of the large dosage levels used. The failure to obtain favorable results in the first two investigations could have been predicted. It is unfortunate that weight considerations were not taken into account in determining the doses to be used and it would be worth repeating this work using stimulating levels. The reduced incidence in spontaneous mammary cancer may have been a nonspecific anti-serum reaction on the virus milk factor and may not have been directly relevant to ACS screening.

Davis (44) treated 106 patients with ACS prepared by Goldblatt administered in doses of 0.05, 0.1, and 0.15 ml. of the serum diluted 1 to 10, over a period of seven days. This corresponds to about 0.4 BU/kg which is the middle of the stimulating range and hence probably less effective than a lower dosage would have been. In 62 cancer patients so treated, marked relief of pain was reported in 10 cases along with increased weight, better appetite and a general sense of well being. In eleven cases only moderate benefits were noted. In one case a dramatic recession of a cervical carcinoma occurred after five courses of injections.

With the exception of Movitz's work, the screening of ACS for its effect on malignancy has been very unsatisfactory in both design and content. The use of ACS in blocking doses and a failure to realize that the possible values of the preparation lie in its use as an adjunct and not as a primary therapeutic means must both be overcome in future work before the place of ACS in cancer therapy can be properly evaluated.

e. Arthritis.

In the translation of one of Bogomolets' reports (25) the statement is made that ACS was found beneficial in "acute rheumatism." Immediate interest was generated in this country because "acute rheumatism" was generally interpreted as arthritis—one of our major medical enigmas for which no fully successful treatment, not even ACTH nor cortisone, has as yet been developed. The consequence of this interest was a series

of investigations designed to evaluate the possible clinical application of ACS as a therapeutic agent in arthritis in its various forms.

Davis (44) reported pronounced relief in 12 patients with rheumatoid arthritis. He used Goldblatt's ACS preparation at a dosage level of about 0.4 BU/kg which is somewhat high for maximum stimulating effect. Rogoff, Freyberg, Powell and Rice (136) used ACS, also prepared by Goldblatt, on polyarthritis induced in rats by organisms similar to those which produce rat pleuropneumonia. Three injections of ACS, 0.05 to 0.15 BU/kg, prior to and simultaneous to the inoculation of the infecting organisms demonstrated neither prophylactic nor therapeutic effects. Movitz et al. (115) suggested that the doses were too small, but this is a moot question for this syndrome. Rogoff et al. (136) reported further than 29 clinical patients in various stages of rheumatoid arthritis and ankylosing spondylitis experienced only sporadic relief when ACS was administered. In this group of cases, dosage series of 0.5, 1.0 and 1.5 ml; and of 0.3, 0.6 and 0.9–1.0 ml were used. (Goldblatt's preparation, C. T., 1:100 or greater). These two series correspond to total doses of 0.4 and 0.25 BU/kg respectively assuming that diluted serum was used, or to doses of ten times these amounts if the serum was undiluted. The article is not specific on this point.

Boots, Coss and Ragan (34) treated over fifty patients with ACS prepared by Goldblatt. The dose levels were not stated but were probably similar to those used by Davis (44). About a fifth of the patients were benefited by the treatment, but relapses occurred as found by Rogoff et al. (136) and Bach (9) in England.

Solomon and Stecher (142) used Goldblatt's ACS on a three dose schedule of 0.5, 1.0 and 1.5 ml (presumably diluted serum) on twenty patients with rheumatoid arthritis and on twenty-four with degenerative joint disease. In no case was any permanent relief obtained.

Granirer (63) using ACS prepared by Wyeth (C. T., 1:100) in a dosage series of 0.05, 0.10 and 0.15 ml (or about 0 4 BU/kg) treated several patients with osteoarthritis (ages 49 to 62). Only two cases showed any marked improvement at the end of a

year. Granirer pointed out that almost any therapy in arthritis gives about 20% improvement.

A carefully considered investigation by Kling (86) not only reported ACS to have very little, if any, value in the treatment of arthritis but may also have furnished a clue to the understanding of the whole problem. Quoting,

"the imposing list of diseases claimed by Bogomolets and his co-workers to have been benefited by the anti-reticular cytotoxic serum (ACS) includes also acute rheumatism. It is to be regretted that the translators were not aware that in part of the European literature this term indicates rheumatic fever. That the latter was meant by the Russian authors is evident by the qualification that anti-reticular cytotoxic serum is contraindicated in patients who suffer from endocarditis and myocarditis."

Kling (86) used serum from Wyeth and also from Strauss of the Cedars of Lebanon Hospital in Los Angeles. The original Bogomolets dosage schedule of 0.05, 0.10, and 0.15 ml of serum was used as well as the revised schedule developed by Strauss and his associates (155) which calls initially for 0.02 ml twice weekly with the dose subsequently increased by 0.02 ml at each subsequent injection to a maximum of 0.2 or 0.25 ml by the end of a six week period. These schedules correspond to total doses of 0.4 BU/kg which is in the high range for human stimulation for a period of about 20 days. In 30 cases of rheumatoid arthritis, 20 of osteoarthritis, and 10 of fibrositis only 23% were improved to any degree. Kling also summarzied and analyzed the work of Bach in England (9) who administered to 35 patients, Rogoff et al. (136), Boots (34), and his own work. This represents a total of 128 patients, a large enough sample to yield at least an indication of the therapeutic value of any preparation. In all cases over 50% relapsed after short periods. The conclusion may be reached that ACS has little, if any, use in the treatment of the arthritic type of disease.

f. Miscellaneous screening.

Dermatology.

Friedman and Stritzler (58) used ACS prepared by Goldblatt in a total dose of about 0.4 BU/kg in a series of three graded injections as previously described. It failed to halt or cure a

severe case of Mycosis fungoides. In this case, nitrogen mustard, which had been given before the ACS, also failed. The patient died. However, Samitz and Stritzler (137) reported some improvement in lymphoblastomas, including Mycosis fungoides, using Goldblatt's serum according to the same dosage schedule as above. They reported failures in exfoliative dermatitis (one case), generalized psoriasis (one case), and pemphigus (six cases). Beneficial effects were reported in four cases of chronic leg ulcer. Malisoff (94) also reported favorable effects in chronic skin ulcers and other types of ulcers of long standing. Mashkilleison (103) claimed successful results in the treatment of chronic skin ulcers with ACS and made the observation that better relative healing rates were obtained with older as compared with younger patients.

Tuberculosis.

The use of ACS in non-exudative tuberculosis was recommended by various Russian investigators (73, 92). One paper has appeared in this country by Nayer (116) on pulmonary tuberculosis treatment with ACS. Nayer used serum prepared by Goldblatt in the standard series of injections on the first, fourth and seventh days in doses of 0.5, 1.0 and 1.5 of 1:10 dilution. On the basis of Goldblatt's serum, this is about 0.4 BU/kg, a high dosage level for the best results.

Nayer kept and used the diluted serum for the third injection. This is not recommended practice because of the tendency of ACS to decompose rapidly after dilution and is mentioned not as a criticism of Nayer's work but as a guide for the future. Of the ten patients Nayer treated, no beneficial effects were observed either by X-ray or clinical examination.

Atrophic Rhinitis (Ozena).

The Russian investigators reported successful treatments with ACS in this syndrome. Jackson (75) using ACS prepared by Goldblatt at his recommended levels of a calculated 0.4 BU/kg in a graded series obtained very poor results in 20 cases of ozena and four cases of nasopharyngitis. The subjective data indicated some improvement such as reduced headache and other symptoms, but very little objective evidence of improvement was noted.

Otolaryngology.

Steinberg (145) conducted a series of investigations on children using Goldblatt's serum. His cases included various syndromes of the upper respiratory tract such as allergic rhinitis, tonsillitis (both acute and chronic), etc. A series of 0.25, 0.5, and 0.75 ml of diluted serum was used. On a basis of 1:100 minimum titer and assuming an average weight of 13 kg (the majority of the patients were two years and under), this is equivalent to a dosage level of about 1.0 Bu/kg, which is quite high and above the optimum stimulating dose. The results were variable, as might be expected from the dosage level employed.

Anti-Reticular Cytotoxic Serum and the Aging Problem

It is a rather sad commentary that the one property of ACS that Bogomolets speculated about but never claimed should have received the most publicity on one hand and the least investigation on the other. This refers to the possible value of ACS as an anti-aging factor. Unfortunately, the majority of the American public at all familiar with ACS, and this includes some investigators, think of the preparation as an anti-aging rather than a therapeutic agent. This may be partly due to a very widely disseminated popular article by the dean of American science writers, William L. Laurence (91) which dealt largely with the anti-aging speculations of Bogomolets. It should be understood that Bogomolets never claimed anti-aging benefits from ACS, but that he had speculated about its possible use in this direction on the basis of the hypothecated stimulating action of the serum on the RES.

Bogomolets' book (29), "*The Prolongation of Life*," was widely read in Russian and later translated into English in 1946. As a whole, this work is not consistently scientific and critical in its approach, being written obviously for popular consumption. It does include considerable speculation regarding the actions of ACS on the RES which speculation is essentially a restatement of Bogomolets' views on the subject which are more detailed in his technical papers. Further investigative work must be done to establish either the validity or lack of validity of these views before any *a priori* evaluation of ACS as an anti-aging factor is possible. On the other hand,

it is certainly true that the possible application of ACS in this direction may be explored *empirically* without consideration being given to the justice of Bogomolets' premises. The use of ACS for gerontotherapeutic purposes in Russia, at least up to the time that scientific data from that country was still available, had been investigated to a much lesser degree than the value of the serum for other therapeutic applications.

Gatsanyuk (60) reported that a single injection of ACS at a dosage level of 0.01 ml in senile rabbits (about 0.4 BU/kg) decreased the cholesterol concentration in the blood and muscles, increased it in the brain and did not affect it in the lungs, liver or kidneys. A series of three injections at two to three days apart decreased the cholesterol content in all organs. Further, the single injection caused an increase of fatty acids in most organs except the lungs, while the three injection series increased the fatty acid content in all organs without exception. Also, the single injection increased the water content of the blood and liver, decreased it in the kidneys and brain, and left it unchanged in the muscles and lungs. The three injection series increased the water content in the lungs and liver, decreased it in the muscles and left it unchanged in the brain, kidneys and blood.

Medvedeva (108) and Novikova-Dantsiger (119) reported significant changes in the tissues of old animals following the administration of ACS. Medvedeva also investigated (106) the effects of prolonged stimulation at two month intervals for six months on the tissues of rabbits. Constant and pronounced changes in protein composition and nitrogen content were found. Bogomolets (29) and Rochlina (135) discussed possible anti-aging uses of ACS.

Contrasted with the paucity of accounts from Russia of controlled laboratoy investigations of the gerontotherapeutic values of ACS, many reports from that country indicate the wide use of ACS in the attempt to rejuvenate old patients. However, these reports also state in many cases these patients did not fare too well. This in itself, though, neither proves nor disproves the validity of the case because, judging by published indications, the Russian investigators have been using too high dosage levels and have not sufficiently examined the problem

of optimum dose frequency. In addition to this, it may be expected that the stimulating effects of ACS on old patients, when achieved, may even be deleterious in their net results because of additional strain on degenerated cardiovascular systems. In view of the indicated properties of ACS, it may be suggested at this point that it is far more likely to be of value as a gerontotherapeutic or preventive agent insofar as aging is concerned, than a geriatric or remedial agent for the very aged.

It is parenthetically of interest to note that a few single case reports by Malisoff (94), and by Bardach and Sobieski (10) of the Pasteur Institute in Paris indicate favorable responses to its use as an anti-aging factor. However, it must be stressed again that the possible use of ACS in this field cannot be evaluated without thorough and controlled laboratory investigations. No meaningful conclusion can be drawn at the present time.

Discussion

Bogomolets' anti-reticular cytotoxic serum and the framework of theory which surrounds it actually involve two fundamental and distinct types of claims.

First, Bogomolets has developed an hypothesis which extends the concept of the role of the RES in health and disease. According to his contentions, the general state of body physiology is almost directly a function of the condition of the RES. He maintains that the RES is important not only in respect to disease resistance and repair properties of the body, but also that the condition of the RES influences the level of physiological activity of other tissues through controlling the passage of metabolites across capillary boundaries, etc.

Second, Bogomolets has claimed certain therapeutic effects from an anti-organ serum prepared from spleen and bone marrow-cytotoxic in large doses and "stimulating" in small doses. He claims, further, that the beneficial effects of small doses are specifically due to the stimulating effect of ACS on the RES. Further, he maintains that since, according to the first claim, the proper functioning of the RES is concerned with the proper functioning of the body as a whole, then

ACS is automatically useful in a wide variety of therapeutic applications.

These two types of claims should not be confused. It is very difficult, if not impossible, to prove or disprove the first claim, which is a broad hypothesis about certain physiological inter-relationships, except through the slow accumulation of data over many years of work. However, it is easier to verify or fail to verify any specific therapeutic claim, particularly where a specific syndrome is concerned. Further, even the confirmation of all of Bogomolets' therapeutic claims for ACS would not in themselves establish the validity of his hypothesis regarding the role of the RES. It would merely serve to lend it possible inferential support. On the other hand, if his hypothesis should ultimately fall, this would not suffice to disprove the value of ACS as a therapeutic agent. If the hypothesis should be disproved, but the therapeutic claims for ACS stand, then this would establish only that its mode of action has been entirely misunderstood and that some other explanation of its action must be advanced.

Actually, so far the proof or disproof of the two types of claims are not clear cut. For example, the work of Pomerat, Anigstein and their co-workers has gone far to support the basic theories of the physiological actions of ACS as claimed by Bogomolets and his groups. Both tissue culture techniques and animal experiments were used to demonstrate the cytotoxic and stimulating effects of ACS and there is nothing in the evidence that violently contradicts Bogomolets' assumption about the role of the RES. In some respects, the evidence may even be interpreted, without straining, to support it. The fundamental approach of Anigstein and Pomerat has been very successful in the screening of ACS. Their work under specific and controlled conditions illustrates the action of ACS as an example of the general class of anti-organ sera. The fact that Malisoff and his collaborators (94, 95, 96) established that spleen antigen alone was sufficient for the preparation of ACS does not in itself either negate or support the basic claims of Bogomolets.

Miale (110) has verified the increase of monocytes on small dose stimulation and the depressing effects of large doses of

spleen ACS. The cytotoxic effects in wound healing were verified by Nickerson (117). The stimulating effects of small doses on wound healing were supported by Malisoff and his associates (94, 95, 96) and by Maltz and his group (97). Both the cytotoxic and stimulating effects of small doses on the fracture healing rates of rabbits (148) and the stimulation of the healing rate in clinical patients have been verified by Strauss et al. (155) and by Pomerat et al. (138). The inhibition of rickettsia by ACS has been demonstrated by Anigstein and his group (6, 7, 8). The ineffectiveness of large doses of ACS on Hodgkins Disease was reported by Abernethy and his collaborators (1), and the beneficial effects of small stimulating doses by Malisoff and his group (94, 95, 140). The decrease of pain in inoperable cancer was confirmed by Goldblatt (61) and Davis (44). The lack of value of large doses was shown by Heiman and Meisel (66, 67, 68) and the suppression of one type of cancer, the Brown-Pearce carcinoma in rabbits was demonstrated by Movitz and his co-workers (115). However, it should be recognized that as a whole, the entire problem of the therapeutic screening of ACS in cancer has been neither extensive nor carefully planned. Further work in this direction will be necessary before the true worth of ACS in the treatment of malignancy can be evaluated.

Extensive screening of ACS in arthritis (34, 63, 86, 136) has conclusively proved that the preparation has very little, if any, use in this group of diseases. This early work is in agreement with more recent investigations that suggest that arthritis may be due to an hormonal imbalance, or even nutritional deficiency. If the former conclusion is valid, the proper approach to the arthritis problem may well be along strictly endocrinological paths and ACS would be of very little value. Furthermore, Kling (86) has shown that the interest originally generated in ACS as an arthritic remedy was the result of a mistranslation of a Russian report.

Inconclusive results were obtained in some dermatological conditions (137), and positive results in a few cases of skin ulcers (94). The Russian claim for the value of ACS in the treatment of skin ulcers requires further investigative work before any final statement can be made.

Nayer's (116) single investigation of ACS in tuberculosis was negative in its results. However, the dosage level used was probably too large for optimum stimulating results. Negative results with ozena (75) were also associated with the use of a too high dosage level.

The use of ACS as a gerontotherapeutic agent has not been systematically investigated in this country at all, and only slightly abroad. A few clinical cases (94) indicated favorable results, but these are insufficient to permit any general conclusions to be drawn. Investigations of ACS as an anti-aging factor are essential before any generalization about its use in this field can be valid. Favorable results have been reported in France (10) in elderly patients using spleen tissue as the antigen for the preparation of ACS. Details of this work have not been made available to date.

The use of semi-blocking dosage levels of ACS by some investigators, and particularly the adoption of the higher levels recommended by Marchuk (100), rather than the lower doses recommended by Bogomolets (25) is partly due to the development of what has been called the "penicillin attitude of mind." This means that *if some is good, more is better.* In the case of ACS, however, this view is certainly incorrect and has seriously handicapped proper screening of the preparation.

It is recommended that no ACS screening program be initiated for any syndrome unless the proper stimulating dosage level is used, unless the actual titer of the serum is known at the *time of use*, unless the weight of the patient or animal is taken into account, and, preferably, unless the investigator is somewhat familiar with the background of the use of anti-organ sera. This will probably help to prevent misleading data and incorrectly drawn conclusions from confusing the field. It is essential that a reliable serum of known potency be used in any ACS screening program.

Further claims made by Russian investigators for the use of ACS such as frostbite (92), peptic ulcer (122, 88), ulcerative cicatrix (12), abscesses (25, 112), ocular tuberculosis (73), hypertension (19, 25), septic angina (2, 81, 83), schizophrenia (22, 23, 133), gynecological and obstetrical conditions (139), gastro-intestinal diseases (82), typhoid (157), pneumonia and

brucellosis (124), scarlet fever (15, 25), malaria (50), tonsillitis, eczema, generalized infections and other diseases (92), have not been investigated in this country.

It becomes evident that the systematic screening of ACS has only begun. However, in many instances, when proper dosage levels have been employed, quite favorable results have followed. Also, it must be emphasized again that the practical application of ACS as a therapeutic material is to a large extent that of an adjunct to standard and accepted treatments with which it in no way interferes.

Should further screening support the claims, or enough of the claims, of the Russian investigators, then it is to be expected that ACS may ultimately appear as a standard and generally accepted therapeutic agent and may even ultimately play a significant role in gerontotherapeutics.

At the present time, however, it is quite impossible, on the basis of available evidence, to predict the future of the preparation and its possible applications.

Summary

Bogomolets' anti-reticular cytotoxic serum (ACS) has been only partially screened in America. The action of ACS, both cytotoxic for large doses and stimulating for small doses, have been verified by tissue culture techniques. The stimulatory effects in wound and fracture healing have been verified. The relief of pain in human cancer and the suppression of the Brown-Pearce Carcinoma in the rabbit have been demonstrated. The suppression of rickettsia in animals has been verified.

Improvements in the preparation of the serum have been accomplished and the optimum stimulating dosage level has been established at 0.1–0.15 BU/kg as given in a single dose repeated about every three weeks in man, and two to three weeks for mice, rats, dogs, guinea pigs and rabbits.

ACS has been conclusively demonstrated to have very little, if any, value in the treatment of arthritis. The possible use of ACS as an anti-aging agent has not been investigated in America.

Bogomolets' hypothesis about the role of the RES has been neither proved nor disproved. However, the possible practical clinical value of ACS does not depend upon the strength of this hypothesis. It may be established or refuted independently.

Further screening and general investigation is recommended to enlarge the possible field of value of ACS and to facilitate the practical development and production of the serum should this be warranted on the basis of successful clinical trials.

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FAT IN THE DIET IN RELATION TO NUTRITION OF THE DOG

I. Characteristic Appearance and Gross Changes of Animals Fed Diets With and Without Fat*

Arild E. Hansen and Hilda F. Wiese

In an attempt to determine the role of dietary fat in human nutrition it was found necessary first to establish more fundamental facts than are known regarding the lack of fat in the diet. For this purpose experimental animals have been maintained on regimens containing various amounts and kinds of fat. In the work reported herein, the dog has been used as the experimental animal inasmuch as it was felt that periodic lipid analyses of the blood and tissues were essential for correlation with any gross changes which might develop on the various diets (Hansen and Wiese, 1943; Hansen, Wiese and v. Miller, 1944; Wiese and Hansen, 1944). Relatively large amounts of blood and skin can be taken frequently from the dog. This was considered important because of the relationships which had been observed previously between the serum lipids and the clinical condition of eczema and between the change in the composition of the serum lipids and the appearance of the skin after the addition of certain fats to the diet of these patients (Hansen et al., 1933, 1937, 1947).

Experimental results covering various phases of the general subject of the effect of dietary fat in relation to nutrition of the dog will be presented in a series of four papers covering the following subjects:

I. Characteristic appearance and gross changes of animals fed diets with and without fat.
II. Lipid composition of tissues from animals fed diets with and without fat.
III. Spectral analysis for unsaturated fatty acid content of tissues from animals fed diets with and without fat.

*From the Department of Pediatrics, The University of Texas Medical Branch, Galveston, Texas. Support for the work herein reported has been made by grants-in-aid by the National Live Stock and Meat Board and a contract sponsored by the Bureau of Human Nutrition and Home Economics, United States Department of Agriculture and authorized by the Research and Marketing Act. Received for publication May 31, 1951.

IV. Histologic features of skin from animals fed diets with and without fat.

Material and Methods

In the experimental work with dogs it was soon learned that the most marked effect of a diet low in fat was manifested by changes in the hair and skin of the animals. The alterations developed very much sooner in young puppies than in adult dogs. Hence it was found advantageous to start with puppies at the time of weaning or shortly thereafter. Mongrel puppies which had been freed from intestinal and skin parasites were used in all of the experiments reported. Each animal was housed in a separate wire cage.

The basic low fat diet has the following caloric composition:

Protein (casein, skim milk powder)	23.6 per cent
Carbohydrate (sucrose)	75.5 per cent
Fat (milk fat)	0.9 per cent

The ingredients used to provide 100 calories were:

Casein	4.5 grams
Skim milk powder	5.0 grams
Sucrose	15.0 grams

Sufficient water to give a palatable mixture was added to the dry ingredients.

The fat in the diet is derived from the skim milk powder and casein. The amount was determined by alkaline extraction with alcohol, ethyl and petroleum ether (Official Methods, 1950). No attempt has been made to use vitamin and lipid extracted skim milk powder or casein. In addition, all animals regardless of the diet were given the following daily supplements: celluflour, 1 tablespoonful; artificial bone ash mixture and Cowgill's salt mixture 0.2 g/kg body weight (or U.S.P. salt mixture No. 2, 0.4 g/kg); vitamin A (concentrate in natural ester form) 1600 units; vitamin B complex (Ribranex*) sufficient to supply individual factors in quantities recommended by Schaeffer, McKibbin and Elvehjem (1941); vitamin D (Drisdol) 250 units; and the weekly administration

*Generously furnished by Wyeth, Incorporated.

of vitamin E (Abbotts, mixed tocopherol) 25 mg equivalent to 15 mg alpha tocopherol. A large number of puppies have been maintained on this diet which provides about one per cent of the caloric intake as fat. The weights were recorded periodically and changes in the gross appearance of the animals were followed graphically as illustrated in Figure 1.

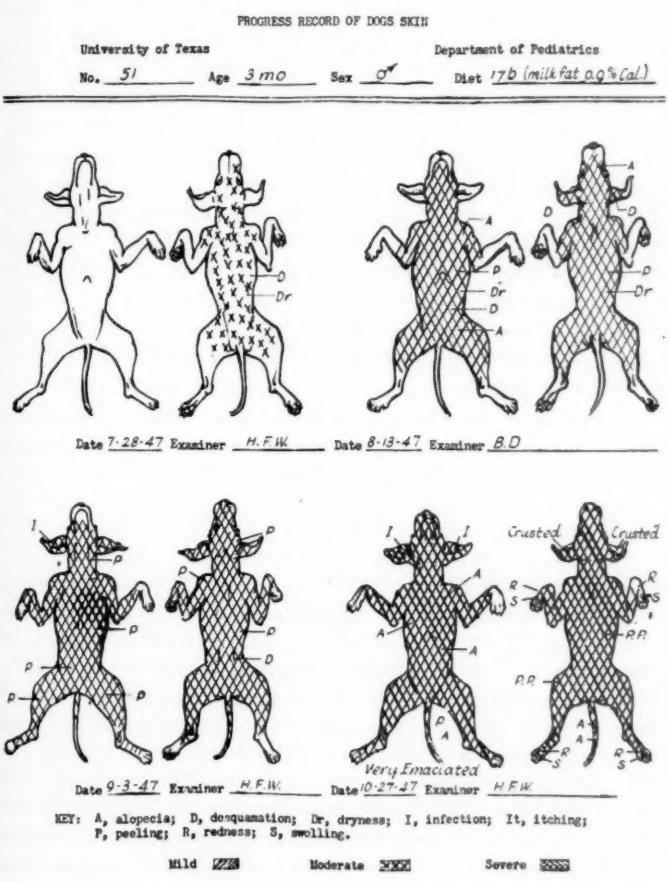

PROGRESS RECORD OF DOGS SKIN
University of Texas
Department of Pediatrics
No. 51 Age 3 mo Sex ♂ Diet 17b (milk fat 0.9% Cal.)
Date 7-28-47 Examiner H.F.W.
Date 8-13-47 Examiner B.D
Date 9-3-47 Examiner H.F.W.
Date 10-27-47 Examiner H.F.W.
Crusted
Crusted
Very Emaciated
KEY: A, alopecia; D, desquamation; Dr, dryness; I, infection; It, itching; P, peeling; R, redness; S, swelling.
Mild
Moderate
Severe

FIG. 1. Graphic representation of periodic evaluation of appearance of animal.

Routine blood and urine analyses, which will be described later, as well as plasma protein and vitamin A levels were done according to standard procedures.

Other animals were maintained on diets containing 6, 11, 16 and 30 per cent of the calories as fat. Since the basic diet contains about one per cent of the calories as fat when 5, 10, 15 or 29 per cent of the calories as sucrose are substituted isocalorically by fat, the total fat intake then constitutes 6, 11, 16 or 30 per cent of the calories. The kinds of fat used were lard, baconfat drippings, butterfat and a commercial vegetable shortening. In some instances, these diets were substituted for the low fat regimen after typical skin changes had developed on the latter diet. The methyl ester of linoleic acid was fed at a caloric level of one per cent to two dogs and the ethyl ester of arachidonic acid to one dog. All the diets were fed on a caloric basis, providing an intake of 150–160 cal/kg body weight for puppies up to about three months of age. This was gradually decreased to about 80 cal/kg by the time the animal reached maturity. Adult animals received 40–50 cal/kg body weight.

A total of 70 dogs have been under observation. The results reported are on 53 animals on which careful and detailed observations and analyses have been made.

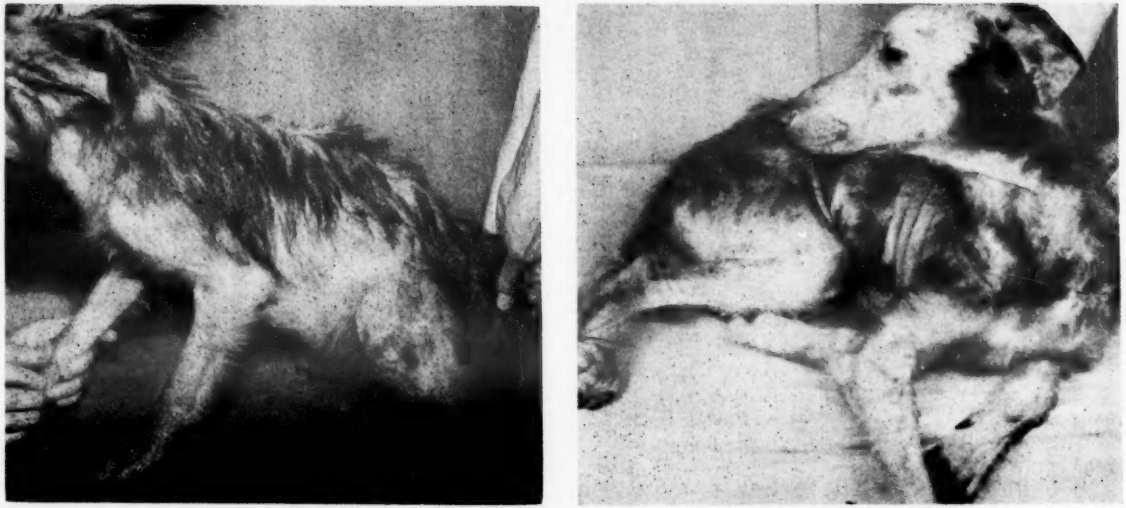
Results

Dogs Maintained on Low Fat Diet

The gross changes in the appearance of young puppies maintained on a diet containing one per cent of its calories as fat are characterized first by dryness of the skin and hair, followed by a fine desquamation which becomes progressively severe with extensive loss of hair. There may be numerous small areas of infection, some impetigo-like in character, on the ventral surface, back of the ears and on the dorsal surface of the skin. At this stage, apparently there is considerable itching as evidenced by scratching. The desquamation becomes increasingly severe so that large areas of epidermis can be peeled off and the paws are reddened, edematous and often show oozing between the toes. After prolonged deficiency (several years) the skin tissue

in the region of the leg joints and paws may show lichenification. Frequently a purulent discharge exudes from the ear canals. Gradually, there is generalized redness of the skin, marked alopecia and emaciation. When alopecia occurs, the skin often looks and feels greasy. Some of these changes are illustrated in Figures 2–4. The rate at which these abnormalities develop varies considerably. Some litters of young puppies go through the entire cycle in a period of three to four months. In other instances, severe changes do not develop until the animals have been maintained on the diet for 12 to 18 months (Figure 3). Dryness of the hair and a fine flakiness of the skin will be evident in about three months but the alopecia, edema and emaciation may not appear for 1 to 2 years. Thirty-two animals have been on this regimen for 1 to 4 years. One dog was maintained on the low fat diet for six years. When animals are on the fat deficient diet for prolonged periods, they go through cycles in which there is extensive desquamation with almost complete loss of hair followed by a period in which the desquamation is decreased markedly and new hair appears. Changes in the appearance of the skin and hair have been noted with animals both in a temperate and semi-tropical climate and under various degrees of humidity. Severe alopecia and emaciation were not obeserved in the temperate climate. Part of this difference may be dependent on the previous history of those animals. However, all of the marked dermatologic effects have been observed in a humid climate. In addition to the abnormalities of the hair and skin all the dogs have shown a change in temperament. First, they become very excitable and later tremulous. Some of the animals appear to succumb quite readily to infection.

The increased susceptibility to infection which is a constant finding is not confined to infected and impetigo-like areas on the skin. Although these lesions were noted in 20 of 39 animals (51 per cent) on the low fat diet and in only 4 out of 17 animals (23 per cent) receiving 30 per cent of their calories as fat, the development of a continuous purulent discharge from the ears of dogs receiving one per cent caloric fat has appeared even more significant. This has been noted in 23 out of 39 animals (59 per cent) and has never developed in dogs receiving 6 per cent or more of their calories as fat. A cessation of the running



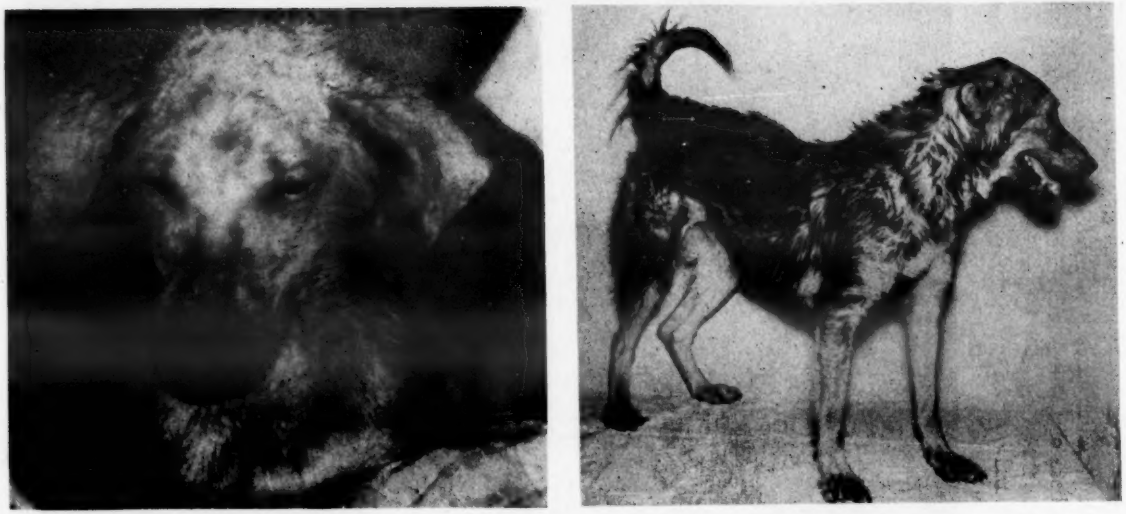
A. Age 7 months.

B. Age 9 months.

FIG. 2. Dog No. 47, low fat diet.

from the ears occurred when as small an amount as 5 per cent of lard or butterfat was substituted isocalorically for sucrose in the diet. Bacterial cultures of the material in the auditory canal of animals both on the low fat and control diets showed that no characteristic flora existed in either group. Pseudomonas aeruginosa was found only in the discharge from the ears of some of

FIG. 3. Dog No. 42, low fat diet, 7 months, marked desquamation.



A. Low fat diet 7 months.

B. 5% fat 3 months. Note oily appearance of hair.

FIG. 4. Dog No. 46.

the animals on the low fat diet although it was not found consistently. On one occasion during a diarrheal epidemic of undetermined origin all of the dogs showing diarrheal symptoms were on the low fat diet, 7 of a total of 10 dogs. Two of these dogs died. On another occasion there occurred an endemic characterized by frequent soft stools with blood. Again all six fat deficient animals showed the symptoms for 10–12 days. One died. Only one of the 4 dogs receiving fat had bloody stools for one day.

Of the total group of 53 dogs, 39 have been maintained on the low fat diet. There have been 16 deaths in the total group. With one exception, all the deaths have been in fat free animals. The one exception was a dog receiving 30 per cent caloric fat that died following thyroidectomy. On autopsy the causes of death among the 15 dogs on the low fat regimen were as follows:

2 post-operatively (thyroidectomy)
2 diarrhea
3 infection (2 parasitic)
8 cause unknown except for pulmonary involvement. Extensive consolidation was evident but no bacterial or viral study of the tissue was made. The cardiac and renal tissues appeared normal grossly. Death occurred suddenly. The only symptom was a refusal of food the day preceding death. Anorexia is not a usual symptom among the fat deficient animals.

Seventy-six determinations of hemoglobin were made on the dogs. Animals on the low fat diet were not anemic and showed blood pictures similar to those of the controls. Hemoglobin values were 11 to 23 gm/100 ml; erythrocyte counts, 6 to 7 million/cubic mm; and leucocyte counts, 6,000 to 22,000/cubic mm. The wide range in hemoglobin values seemed to be dependent upon the age of the animal and was not a characteristic of the low fat group. Young puppies had the lower hemoglobin levels. Serum protein values were the same in both groups of animals, 6 to 8 gm/100 ml. Repeated microscopic examination of the urine failed to show evidence of red blood cells. Vitamin A levels* of the serum were determined for nine animals. These

*These determinations were made by Dr. Augusta B. McCoord, University of Rochester, Rochester, New York.

were all high, 183 to 240 micrograms/100 ml and both groups showed the same range.

Typical growth curves for litter mate animals on the low fat and control diets are shown in Figure 5. Because of the great variation in size of the dogs, it is not possible to take average weights for all the animals; however all curves made with litter

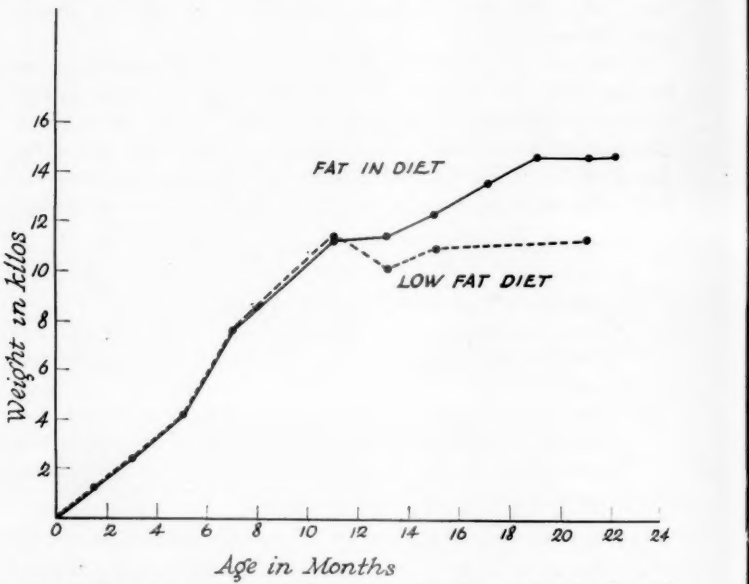


FIG. 5. Growth curves of eight litter mate dogs, four on low fat diet and four receiving 30 per cent calories as fat.

mates show the same trend. There is little or no difference in growth curves of the two groups for the first six to ten months of age.

Control Animals With Fat in Diet

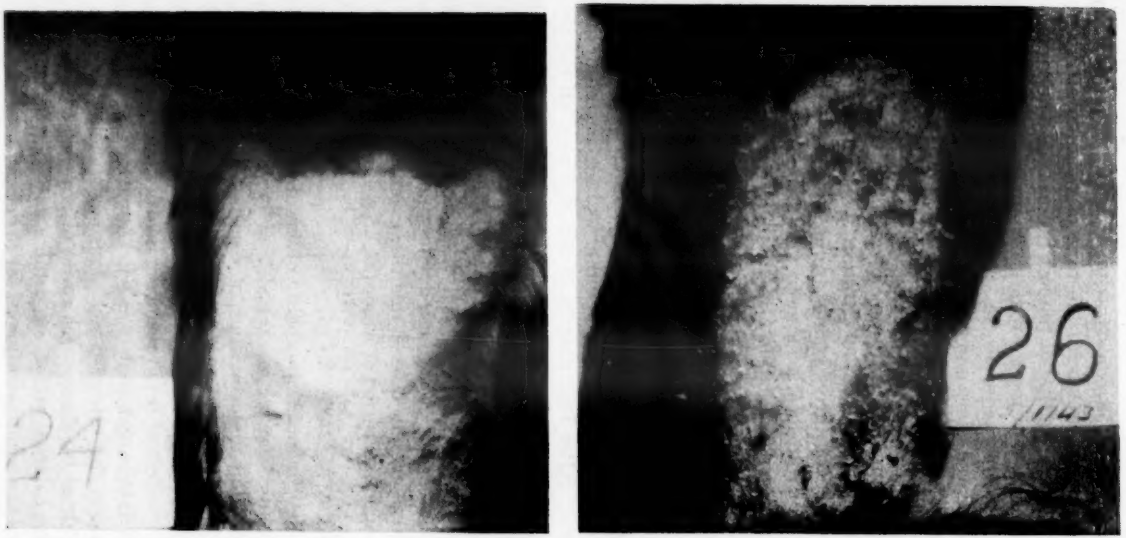
Control animals were maintained on the same low fat diet except for the isocaloric substitution of fat for sucrose. The fat levels were 30 per cent and 6 per cent of the total calories. For comparison, all of these animals had litter mates on the one per cent fat regimen. The types of fat used are indicated below:

Fresh lard, fat at 30 per cent caloric level: Eight dogs were maintained on this diet from the time of weaning at the same total caloric intake as that of the low fat group. The same type of observations regarding growth, gross appearance and laboratory studies were made also on this group. The animals invariably appeared alert and healthy. The skin and hair were smooth and of a soft texture. (See Figure 6.) They did not show the same degree of susceptibility to infection as those of the low fat group. The only type of infection which was manifested was an impetigo-like eruption which appeared on the ventral surface of three young animals. These eruptions responded readily to local treatment. The ears of none of these dogs ever showed the exudate so characteristic for the low fat group, although on culture the same types of bacterial organisms were found in the auditory canals of all the dogs. Diarrhea did not occur in the control dogs receiving 30 per cent fat.

Lard, baconfat, butterfat and crisco, fat at 6 per cent caloric level: A group of four litter mate puppies were placed on a diet having 5 per cent of its calories either as lard, baconfat, butterfat or crisco. After four months of this regimen, all the young dogs were showing evidence of the characteristics of fat deficient animals, desquamation and loss of hair as indicated in Figure 7. A fifth puppy, not a litter mate of the preceding group, also was maintained at a caloric level of 5 per cent fresh lard from the time of weaning. Although dryness and a slight flakiness of the skin were evident after 2 months, extensive loss of hair did not occur during a period of 9 months.

Curative Effects of Dietary Fat

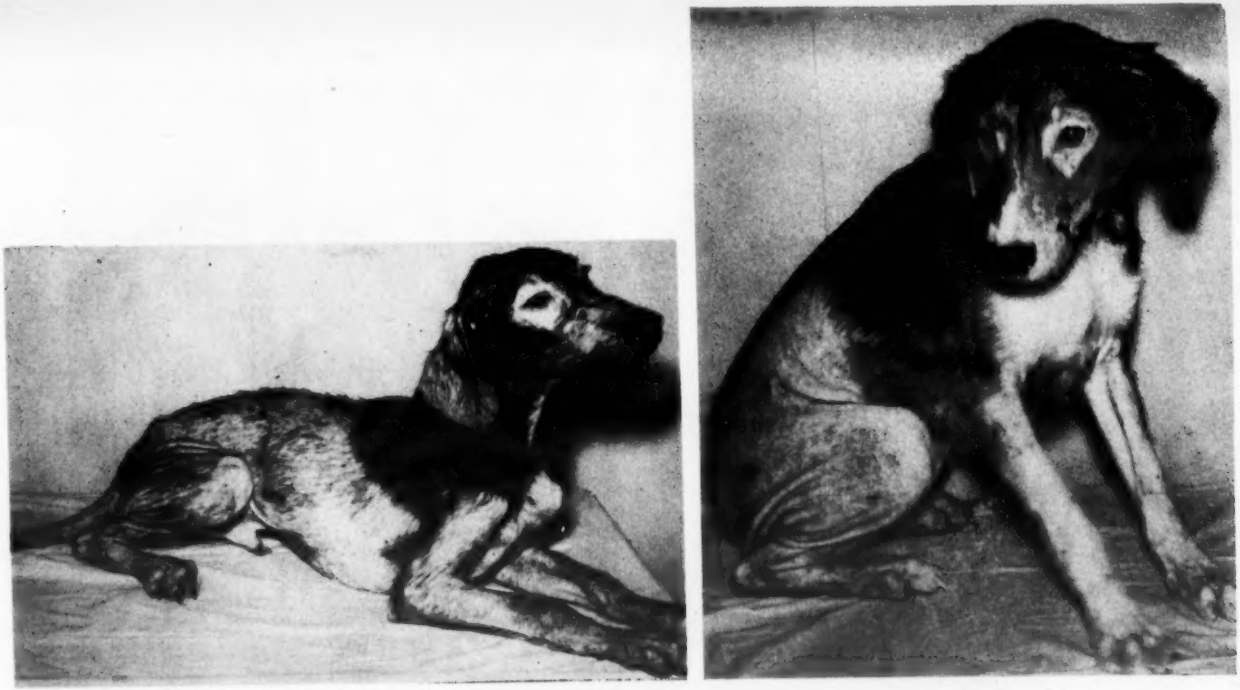
It is evident from the preceding observations that young puppies maintained on fat intakes of 1 or 6 per cent of the total calories develop abnormalities of the skin and hair whereas when fresh lard is fed at a caloric level of 29 per cent (total fat 30 per cent), such abnormalities are prevented. Consequently the curative effect of fat in restoring healthy appearing skin and hair to fat deficient animals was tried at various levels of intake. Differences in the rate at which signs of deficiency develop are well illustrated by the control group of puppies receiving 6 per cent of the calories as fat. A similar variation in the clinical response to the administration of fat to deficient animals may be



A. Control diet.

B. Low fat diet.

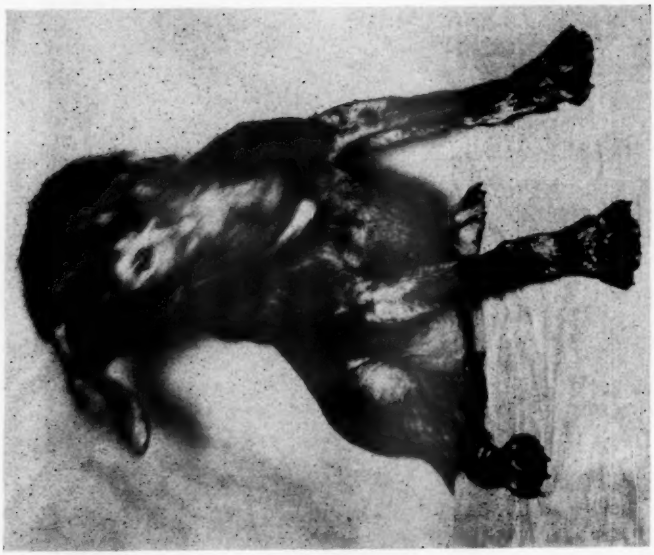
FIG. 6. Close-up of skin of dogs.



A. Lard.

B. Baconfat.

FIG. 7. Dogs maintained from weaning on 5 per cent caloric fat.



D. Crisco.

C. Butterfat.

FIG. 7

expected. Hence, in this phase of the discussion it seems best to describe the results of the effect of various fats in groups, inasmuch as litter mates were used for comparison of one or more kinds of fat whenever possible.

Twenty-nine per cent fat as fresh lard (total fat at 30 per cent caloric level): Four dogs which had been maintained on the low fat diet for 5 months to 6 years and showed marked signs of fat deficiency were given fresh lard, substituted isocalorically for 29 per cent of the sucrose calories. The first change noted was a cessation of the discharge from the ears. This took place in a relatively short time, about 2 weeks, with complete healing in 1 to 2 months. About the same time, there was an increase in the loss of hair, providing alopecia was not complete, and an increase in the amount of desquamation of the skin. However, as layers of old skin peeled off, the new skin which appeared was soft and there was abundant growth of new hair. When the skin still showed fine desquamation the hair often was quite oily. The time required for growth of healthy appearing skin and hair varied depending upon the severity of the depleted state. At the end of 2 to 6 months, the skin and hair of three of the dogs were considered normal grossly. However, nearly 12 months was required for the growth of healthy skin and hair of one dog which had been deficient in fat for six years. A definite change in the temperament of the animals occurred. The excitable, tremulous reaction seen in the fat deficient animals changed to one of alertness and friendliness within a few weeks.

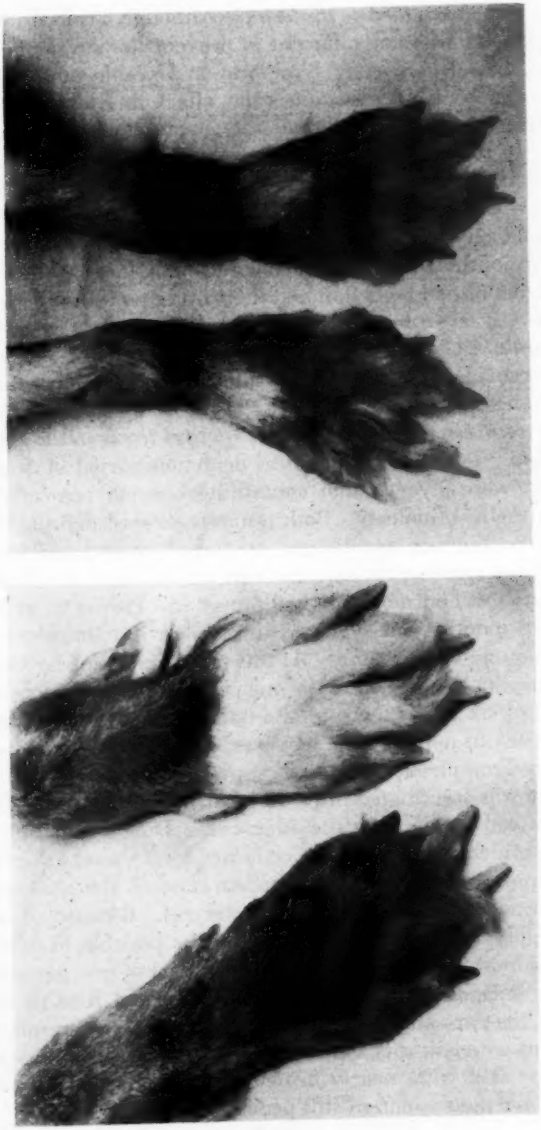
Twenty-nine per cent fat as lard, baconfat, butterfat, or crisco: In the group of four litter mate puppies which showed signs of fat deficiency at a caloric level of 6 per cent, after four months, the fat level was increased to 30 per cent by the isocaloric substitution of lard, baconfat, butterfat or crisco for sucrose (one per cent fat was derived from the basic low fat diet). Within 3 months the skin and hair of all the puppies were definitely improved, although the dog receiving baconfat appeared to have improved the most. In 6 months, all the animals had new complete coats of hair but at the end of 9 months, while still on the same regimens, there was evidence again of dryness of the skin and hair of the dogs receiving lard, butterfat and crisco. These fats were of commercial quality, purchased on the open market.

It was unfortunate that at this time the only lard available for the study was a commercial product containing hydrogenated fat. The composition of this product with respect to unsaturated fatty acids as evidenced by the iodine number of the lard was less than that of a good grade of fresh lard, iodine number of 57 compared to 63–65 for the other lards used in these studies.

Twenty-nine per cent fat as baconfat or butterfat: In a third group of two dogs, both showing severe signs of fat deficiency for 3 years, one was given 29 per cent baconfat and the second was given 29 per cent butterfat. In this experiment more marked differences in the effectiveness of the curative value of baconfat and butterfat were demonstrated than in the preceding group of four dogs. Differences in the appearance of the paws after four months are illustrated in Figure 8. After nine months, the dog receiving baconfat was in very good condition and remained in excellent condition but at the end of 12 months the one receiving butterfat still showed peeling of the skin, coarse hair and reddened paws. After receiving 29 per cent of its calories as butterfat for 2 years, it was quite healthy in appearance.

Fifteen per cent fat as fresh lard: One dog showing extreme effects of the fat deficient diet, after 19 months, was given 15 per cent of its calories as fresh lard. Within five weeks there was a marked improvement in appearance, the numerous infected areas were healing, one ear was completely healed, new hair appeared and the desquamation was lessened. There was progressive improvement of the skin and hair and by 6 months, no desquamation, alopecia or infection was evident. Healthy skin and hair have been maintained at the 15 per cent caloric level of lard for 18 months.

Ten per cent fat as lard: One dog deficient in fat for four years was given a diet containing 10 per cent of the calories as fresh lard. Although the animal showed a slow progressive improvement, at the end of two years his skin and hair were not entirely normal. The ears and feet were in excellent condition and there was good growth of hair over most of the body. However, over part of the dorsal surface coarse stubby hair and infected areas were prominent. There appeared to be extensive itching over this area.



A. Baconfat. B. Butterfat.

FIG. 8. Appearance of paws, 4 months after receiving fat.

Five per cent fat as lard or butterfat: Although a caloric level of 6 per cent fat was not sufficient to prevent the development of some of the fat deficiency symptoms in 5 young puppies, it seemed worth while to ascertain what effect this level would have in severely deficient animals. Two litter mate dogs, deficient for 10 months, were given 5 per cent caloric fat in the form of either fresh lard or butterfat. The rate at which changes in the appearance of the skin and hair took place are best illustrated graphically in Figures 9 and 10. It will be noted that within 3½ months (Figure 9) the dog receiving lard had a complete new coat of hair, soft skin and there were no infected areas whereas it was seven months before the dog receiving butterfat was in a similar condition (Figure 10). Furthermore, it is apparent that this level of fat was not sufficient to maintain either animal in a healthy state.

One per cent essential fatty acid as methyl linoleate or ethyl arachidonate: Two dogs, after a fat depletion period of 5 and 18 months, were given a diet containing one per cent of the calories as methyl linoleate. Both animals showed definite improvement within one month and continued to improve for a period of three months; the ears and feet were in good condition; the skin was soft and new hair had grown in. However, at the end of four months, one dog was again showing considerable desquamation and loss of hair. At this time 5 per cent lard was added to the diet in addition to the one per cent methyl linoleate. At the end of six weeks the skin and hair again showed definite improvement. In neither case, however, could the skin and hair be considered completely healthy in appearance or texture. The experiment was terminated after 18 weeks of the linoleate diet in one case and 24 weeks in the other case. During this time a litter mate which received 29 per cent lard for 24 weeks showed progressive improvement in the appearance of the skin and hair and was considered practically normal. Because of the limited supply of ethyl arachidonate it was possible to follow only one animal for six weeks at a caloric level of one per cent. There was definite improvement in the skin and hair in one month but the ears and feet still showed involvement resulting from the fat deficient diet. At the end of 6 weeks, the infected areas on the skin were nearly healed and there was a full coat of hair. Some desquamation still persisted.

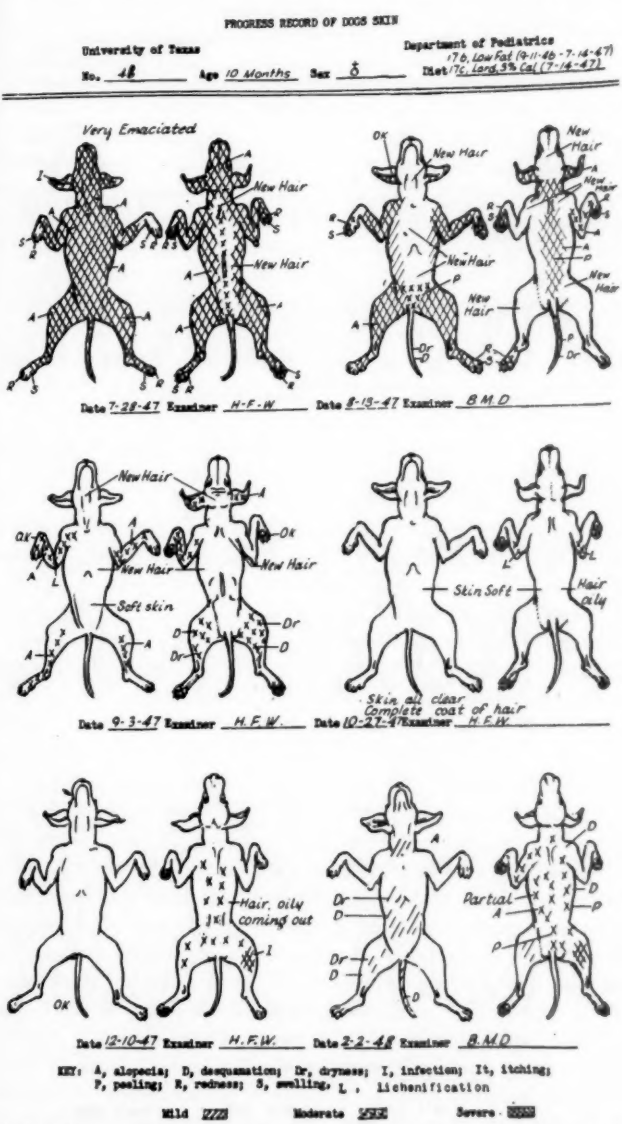


FIG. 9. Diagrammatic representation of changes of skin and hair of dog receiving 5 per cent caloric lard.

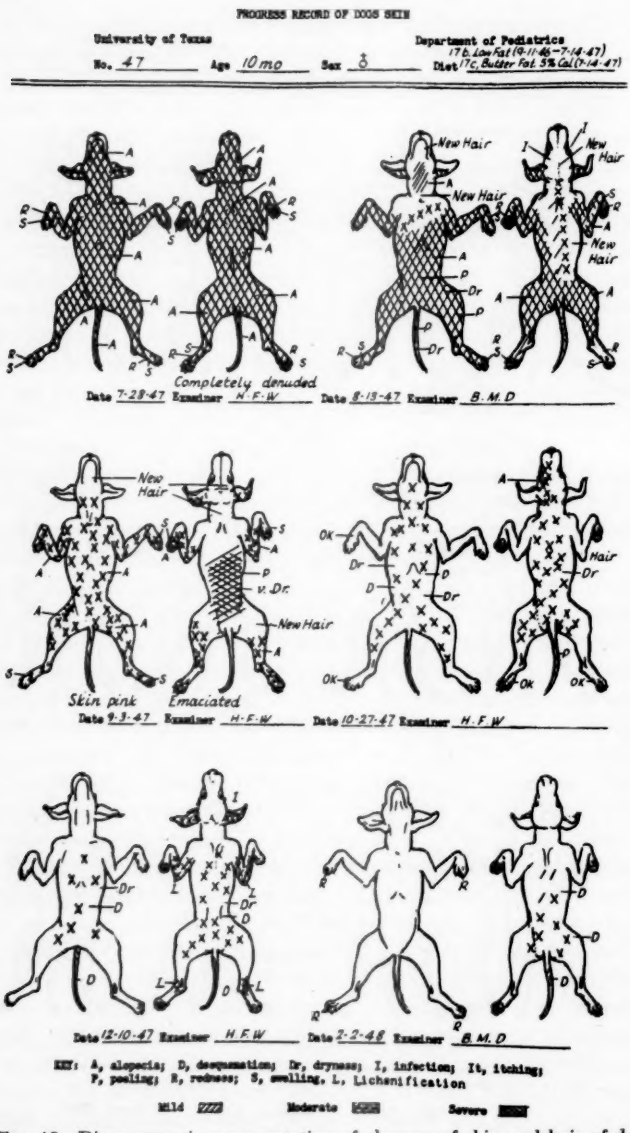

PROGRESS RECORD OF DOGS SKIN
University of Texas
Department of Pediatrics
17 b. Low Fat (9-11-46—7-14-47)
No. 47 Age 10 mo Sex ♂ Diet 17 c. Butter Fat 5% Cal (7-14-47)
New Hair
Completely denuded
Date 7-28-47 Examiner H.F.W
Date 8-18-47 Examiner B.M.D
Skin pink
Emaciated
Date 9-3-47 Examiner H.F.W
Date 10-27-47 Examiner H.F.W
Date 12-10-47 Examiner H.F.W
Date 2-2-48 Examiner B.M.D
KEY: A, alopecia; D, desquamation; Dr, dryness; I, infection; It, itching; P, pealing; R, redness; S, swelling. L, Lichenification
Mild
Moderate
Severe

FIG. 10. Diagrammatic representation of changes of skin and hair of dog receiving 5 per cent caloric butterfat.

Discussion

It is apparent from observations for long periods of time on 53 animals that fat is essential in the diet of the dog. The most marked effects of the absence of dietary fat are 1) development of abnormal skin and hair and, 2) increased susceptibility to infection. Burr and Burr (1929, 1930) first ascribed to fat an essential role for the rat. They described a number of changes associated with the fat deficiency syndrome. These included roughness of the hair, scaliness of the skin, retarded growth, an increased metabolic rate, hematuria, impaired reproduction and early death. The probable cause of death was thought to be due to degeneration of the kidneys. In our experiments with dogs, not all of these abnormalities have been noted to develop. Hematuria and gross degenerative changes of the kidneys which were described originally as characteristic of the fat deficiency syndrome in rats and believed to be responsible for early deaths have not been observed in any of the dogs reared on the low fat regimen. Death in our animals, we believe to be primarily a result of greatly decreased resistance to infection. Of a group of 44 dogs receiving 6 per cent or less of fat 33 (74 per cent) showed one or more types of infection whereas only 4 of 18 animals (22 per cent) receiving 30 per cent caloric fat showed evidence of infection. In the latter group, the only type of infection noted was impetigo-like lesions and these responded readily to local treatment. Of a total of 16 deaths (15 of which occurred in the animals on a low fat regimen) consolidation of the lungs was the only finding in 8 cases at autopsy, and infection of bacterial or parasitic origin was identified in 5 others.

The emaciation observed in the fat deficient animals possibly may be attributed to poor utilization of the carbohydrate and protein resulting from the absence of fat in the diet. A number of investigators working with rats have shown that dietary fat influences the utilization of protein and the general nutrition of the animal (Forbes, Swift, 1944; Forbes, Swift, Elliott, and James, 1946; Forbes, Swift, James, Bratzler and Black, 1946; Bosshardt, Paul, O'Doherty and Barnes, 1946; Scheer, Codie, and Deuel, 1947; Salmon 1947; French, Black and Swift, 1948; Vars 1948). Optimum utilization of protein resulted at a fat level of 20–40 per cent of the diet. Fat at a caloric level of 30

per cent in our experiments was equivalent to about 15 per cent of the diet on the dry basis. The daily protein intakes were: puppies 8.5 gm/kg; young dogs 4.5 gm/kg; adult dogs 2.8 gm/kg. The protein intake even in the absence of fat in the diet appears to be liberal, considerably in excess of the minimum requirement reported by Kade, Phillips and Phillips (1948). In both rats and one human subject (Burr and Burr 1929; Brown, Hansen, Burr, and McQuarrie, 1938) increased metabolism was observed while on low fat diets. Hence, it is possible that the actual requirement for calories is greater in dogs on a similar low fat regimen than it is in animals receiving fat. Metabolic rates were not determined on the dogs. Differences in food consumption, however, do not appear to be a factor inasmuch as all the animals had the same caloric intake regardless of the type of diet and all the dogs regularly consumed their total amount of food. The caloric intake was very liberal for growing animals. All diets were fed on a caloric basis in order to correlate results with those of human subjects, if possible.

Many investigators have confirmed the observations of Burr and co-workers regarding the essential nature and curative effect of linoleic and arachidonic acid in small animals. In our study it was possible to follow the curative effect of methyl linoleate for two dogs over periods of 18 and 24 weeks and the effect of ethyl arachidonate on one dog for a period of 6 weeks. Although definite improvement in the skin and hair was noted in all three animals, complete and permanent cures were not observed at the one per cent caloric level. Certainly at this intake neither the linoleic nor arachidonic acid ester was as effective as 29 per cent lard, baconfat, butterfat or crisco. One may conjecture that increasing the intake of linoleic and arachidonic acid would have resulted in additional improvement, however, this was not possible to ascertain because of the prohibitive cost of such an experiment with dogs. The additional stimulus which was noted with 5 per cent lard to the curative effect of one per cent linoleic acid as well as the results observed with lard, baconfat, butterfat and crisco at various levels indicate that both the kind and amount of dietary fat are of importance in the maintenance of healthy skin and hair. On the basis of 5.6 per cent of linoleic acid in lard (Wiese & Hansen 1951) the animals given 5, 10, 15

and 29 per cent of the calories as lard were receiving respectively, 0.3, 0.6, 0.9 and 1.8 per cent of the calories as linoleic acid. One of the early observations made by Burr and Burr (1930) was that complex unsaturated oils such as corn oil and linseed oil appeared to be more effective as curative substances for the fat deficiency syndrome in rats than single fatty acids. On the basis of our limited studies with fats, there was the following decreasing order of effectiveness in curing the fat deficiency syndrome in dogs: fresh lard, baconfat, butterfat. Only one animal received vegetable oil shortening and the results here were superior to those of butterfat.

A specific attempt was made to follow the effects of lack of dietary fat for long periods of time in a heterogeneous population of dogs, first in order to note the nature and uniformity of the initial effects of the deficiency and, second to ascertain whether or not symptoms other than those observed during the first six months would develop over a prolonged period of time. In studying the therapeutic nutritional value of dietary fat it appears necessary to follow the animals for long periods of time. The first signs of improvement may not be permanent as was noted when one per cent methyl linoleate, 5 per cent lard and 5 per cent butterfat were given to dogs manifesting fat deficiency symptoms.

Summary and Conclusions

Observations made on 53 young dogs for prolonged periods (1 to 6 years) indicate that fat is essential in the diet of the dog. Although death may not occur as a result of a lack of dietary fat marked abnormal changes in the gross appearance of the skin and hair take place. The major changes which developed are characterized by dryness of the skin and hair, desquamation, loss of hair and increased susceptibility to infection. Mortality in the fat-deficient animals was 38 per cent. The syndrome was prevented and cured by the incorporation in the diet of 29 per cent of the calories as fresh lard, baconfat drippings, butterfat or crisco (total calories as fat 30 per cent). Marked improvement was produced when the fat level was increased to 6, 11 or 16 per cent of the calories. However, complete and permanent cures were not observed at levels less than 16 per cent. Incorporation of one per cent of the calories as linoleic or arachidonic

acid ester also resulted in definite improvement in the skin and renewed growth of hair. This level of unsaturated fatty acid alone was not sufficient to effect complete and permanent cures in a period of six months.

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FAT IN THE DIET IN RELATION TO NUTRITION OF THE DOG

II. Lipid Composition of Tissues From Animals Fed Diets With and Without Fat*

Hilda F. Wiese and Arild E. Hansen

It has been demonstrated that when fat is lacking in the diet of the dog, definite abnormalities of the skin and hair develop (Hansen and Wiese, 1943, 1951). In order to see whether or not these gross changes could be correlated with the lipid composition of any tissue, analyses were made of the blood serum, skin, subcutaneous tissue, liver, kidney, and heart of 42 of the dogs maintained under the dietary conditions previously described.

Material and Methods

Blood specimens were drawn after a 20–24 hour fast and hot extractions made of the serum with a 3:1 mixture of alcohol and ether. The alcohol was prepared aldehyde free and the ethyl ether was of anesthetic quality. The following determinations were made on the alcohol-ether extract: total fatty acids by the method of Wilson and Hansen (1936), acetone soluble and acetone insoluble fatty acids by the technique of Hansen (1939), iodine number of the fatty acids by use of the pyridine sulfate dibromide microreagent (Yasuda, 1931), total cholesterol on the unsaponifiable fraction of the total lipid extract using the Lieberman-Burchard color reaction (Sperry and Brand, 1943), and cholesterol esters in the original alcohol—ether extract after precipitation of the free cholesterol with digitonin. In a small group of animals, a separation was made of the serum fatty acids combined as glyceride and cholesterol ester by subjecting the acetone soluble fraction of the total lipids to enzymatic hydrolysis with castor bean lipase (Kelsey, 1939).

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In preparing skin for lipid analysis, the hair was first removed by clipping closely with scissors. The skin specimens were usually taken from the outer surface of the thigh, either by excision under local anaesthesia (with procain) or at the time of autopsy. The area removed was dissected along the fascial layer and any remnants of subcutaneous fat were carefully trimmed away. Subcutaneous fat for analysis was obtained at the same time. After weighing, the specimens were covered with aldehyde-free alcohol and allowed to stand refrigerated overnight. They were then ground in a mortar with sea sand and hot extractions made with the 3:1 mixture of alcohol-ether used for the serum. The total fatty acids, iodine number, cholesterol and cholesterol esters were determined as for blood serum. Specimens of heart, liver and kidney were taken only at autopsy. They were treated in the same manner as the skin. Analyses were made for total, acetone soluble, and acetone insoluble fatty acids, iodine number of the fatty acids, cholesterol and cholesterol esters.

Results

Serum Lipids

Dogs on low fat diet and control diet containing fat: Results of the lipid analysis of the serum of dogs maintained on the low fat diet and the control diet having 29 per cent of the calories as lard are given in Tables I and II, respectively. The

TABLE I

Serum Lipid Values for Dogs on Low Fat Regimen—Fat One Per Cent of Total Calories

Dog	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F.A.		Acet. Insol. F.A.		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
19	3	321	89.8	142	73.9	155	93.8	158	100
	8	496	95.4	...	...	239	102.4	262	156
	14	491	95.8	198	91.1	137	94.8	263	196
	19	402	94.6	159	93.8	182	93.6	216	149
	23	426	90.5	167	88.4	198	92.8	209	117
	54	331	97.0	128	91.6	130	97.8	175	109
	61	369	96.7	139	94.4	171	98.7	148	124
	65	369	95.6	150	93.1	128	95.2	176	123

TABLE I—(Continued)

Serum Lipid Values for Dogs on Low Fat Regimen—Fat One Per Cent of Total Calories

Dog	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F.A.		Acet. Insol. F.A.		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
20	3	235	81.5	111	76.9	84	84.7	108	62
	5	505	94.7	212	89.3	252	98.6	246	175
	8	528	97.9	...	...	267	98.2	382	212
	13	432	92.3	167	87.2	187	100.0	222	108
	14	462	93.1	171	87.6	218	97.3	229	173
	17	470	89.0	161	91.1	221	90.7	218	150
22	3	372	84.8	184	79.1	148	87.3	160	65
	4	574	92.7	214	90.4	303	96.0	273	193
	7	480	92.3	...	...	233	96.0	261	173
	9	509	93.4	202	88.9	225	94.5	252	170
25	2	374	98.3	153	92.4	175	100.5	173	114
	5	450	94.4	120	89.3	212	103.2	213	132
	11	459	99.1	192	94.4	232	99.2	229	153
	14	404	101.9	168	93.6	212	104.5	212	148
	18	538	103.4	221	99.1	311	101.8	282	201
26	3	354	94.7	143	89.1	182	101.6	162	132
27	7	317	93.5	121	83.0	135	97.0	143	88
32	2	444	94.5	156	85.9	217	97.4	211	137
37	2	370	92.2	156	83.2	172	92.8	174	128
	4	357	95.3	141	89.9	182	100.7	178	113
	6	362	92.3	125	92.9	139	88.3	172	115
	31	364	91.5	120	83.9	143	94.1	136	106
39	24	362	100.0	160	90.9	194	91.7	188	...
	32	400	97.8	160	97.5	223	94.5	231	168
42	15	425	100.8	106	90.8	230	95.9	225	129
	21	394	100.2	142	96.4	218	97.8	187	100
	25	453	95.9	194	90.6	218	93.8	222	142
	35	366	96.7	133	101.3	138	101.7	167	116
	41	445	96.1	164	94.3	149	77.8	181	152
43	11	334	93.4	82	91.0	166	101.3	176	107
	18	336	89.0	126	86.2	145	90.7	162	102
	22	345	85.5	118	93.5	142	86.2	168	89
	31	365	93.8	142	73.0	161	97.1	148	110
46	2	317	92.0	153	84.2	143	98.6	151	88
	5	315	91.5	151	78.8	125	102.8	147	90
	8	325	100.6	164	89.3	154	102.5	149	102
47	2	368	97.3	159	89.5	168	100.6	193	123
	5	349	94.3	129	91.7	171	96.3	178	120
	8	324	98.0	143	99.2	153	88.7	157	111
48	2	355	92.7	...	...	...	...	177	116
49	2	407	95.3	...	...	...	...	196	142
50	2	416	96.4	...	...	...	...	193	141
51	2	385	95.1	...	...	...	...	207	183
	4	322	88.5	135	86.2	160	82.0	172	113
53	2	448	101.8	189	90.2	218	96.8	207	147
	5	464	96.6	180	83.7	236	100.8	195	173
	15	386	84.9	...	...	...	...	117	82

TABLE I—(Continued)

Serum Lipid Values for Dogs on Low Fat Regimen—Fat One Per Cent of Total Calories

Dog	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F.A.		Acet. Insol. F.A.		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
54	2	429	98.2	175	89.5	216	97.6	168	125
	5	510	93.4	177	92.4	251	90.0	202	173
	15	342	90.5	...	...	...	...	115	94
	19	421	93.1	...	...	...	...	146	119
55	2	622	95.8	240	98.1	281	102.3	284	246
	5	464	99.9	179	94.7	172	106.2	227	191
	15	428	92.2	...	...	...	...	154	131
56	2	493	93.8	219	92.6	218	100.8	202	174
	5	452	93.6	158	92.5	201	97.1	200	152
57	2	492	93.5	197	92.4	198	93.0	213	159
58	3	237	85.0	...	...	...	...	86	58
59	3	424	94.0	...	...	...	...	115	94
	5	368	93.9	159	92.2	154	95.0	128	118
	8	544	91.9	...	...	...	...	...	...
	11	394	96.5	...	...	...	...	155	119
60	3	327	85.7	...	...	...	...	114	...
	5	512	84.6	191	85.9	188	85.7	175	151
61	3	389	87.1	...	...	...	...	142	71
63	3	333	90.5	...	...	...	...	138	72
66	4	494	97.8	...	...	...	...	...	...
67	4	432	90.7	...	...	...	...	...	...
68	4	459	101.7	...	...	...	...	...	...
69	4	606	94.7	...	...	...	...	...	...
70	4	604	93.6	...	...	...	...	...	...
Mean		412	94.0	160	89.9	189	95.9	189	131
SD		80	4.4	31	5.7	46	5.6	50	38
SE_M		± 9.0	± 0.49	± 4.1	± 0.77	± 5.9	± 0.74	± 5.9	± 4.4

TABLE II

Serum Lipid Values for Control Dogs with Lard in Diet—Fat Thirty Per Cent of Total Calories

Dog	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F.A.		Acet. Insol. F.A.		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
15	3	416	100.8	204	97.4	185	104.7	227	173
	4	658	117.1	275	117.6	322	115.7	389	308
	5	612	123.3	277	126.9	325	116.8	412	283
	7	530	117.9	...	....	207	120.0	362	266
	13	563	110.8	224	110.7	237	111.9	324	197
	22	504	107.2	198	116.0	280	92.4	275	187
16	3	409	115.5	184	117.0	199	113.5	210	147
	8	510	116.6	156	....	219	114.9	309	180
	10	501	113.5	219	118.0	201	110.5	280	166
17	3	463	98.8	208	97.3	213	98.3	246	174
	12	542	108.4	218	107.5	218	107.8	297	210
	17	414	105.7	163	106.4	193	105.3	210	152
	22	511	108.9	182	111.8	269	104.0	278	188

TABLE II—(Continued)

Serum Lipid Values for Control Dogs with Lard in Diet—Fat Thirty Per Cent of Total Calories

Dog	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F.A.		Acet. Insol. F.A.		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
18	1	461	119.6	221	116.5	197	115.6	278	188
	3	578	115.8	249	116.6	309	112.0	354	258
	5	530	116.8	...	....	237	112.4	346	256
	10	565	108.7	183	112.5	244	107.2	314	227
	17	488	121.7	191	128.2	225	116.4	289	211
	18	572	113.3	214	116.2	281	112.3	320	225
	20	503	110.6	211	114.6	229	106.8	286	204
	27	562	114.2	...	....	...	....	342	236
19	3	454	111.0	169	114.3	211	109.6	198	170
	5	405	102.3	149	104.3	140	91.2	269	152
20	1	412	114.1	156	122.0	248	109.3	224	156
	2	452	107.3	181	103.1	221	104.3	224	156
	2½	531	108.3	224	114.4	276	103.8	298	227
	3	590	106.4	238	109.8	223	105.1	314	233
	3½	554	100.9	255	102.6	161	102.5	294	219
	5	549	107.1	241	108.2	276	98.8	320	226
	11	463	109.8	...	....	...	....	273	173
23	2	446	114.1	171	110.6	220	116.7	187	125
	5	358	115.9	200	121.5	205	112.9	284	198
24	2	403	118.3	128	111.3	192	119.5	191	126
...	6	433	119.0	180	115.0	221	120.0	246	166
	8	480	114.5	194	110.5	225	108.6	277	215
	11	562	112.1	243	118.7	276	111.4	334	233
	12	532	113.2	218	118.2	240	112.3	305	215
	12½	536	113.4	220	119.7	225	111.6	297	217
	14	497	117.5	214	119.9	238	115.5	322	223
	16	536	116.2	243	115.1	289	113.2	306	182
27	2	426	110.7	182	111.5	215	102.4	257	193
	3	390	106.9	171	110.5	178	106.5	244	168
	4	393	112.2	165	109.6	187	112.3	245	176
	6	423	110.7	180	111.9	200	87.2	254	200
	7	405	115.7	161	113.0	197	103.2	245	171
35	2	540	113.9	219	115.2	247	107.1	297	218
	4	564	116.5	223	121.4	273	117.3	330	251
	6	552	115.4	227	120.1	234	104.0	315	249
	30	...	....	107	116.1	280	115.6	257	224
	38	584	109.4	238	102.7	260	110.2	269	161
	42	527	109.4	182	....	232	109.8	339	237
	52	476	115.8	191	117.7	244	102.4	216	177
52	3	430	118.4	169	129.2	229	107.3	224	191
	8	401	110.3	161	115.2	156	104.8	274	116
58	1	334	103.9	155	100.1	147	101.0	117	106
	4	343	103.1	...	....	...	....	...	...
	5	300	102.6	...	....	...	....	...	...
	6	287	106.5	86	101.9	141	99.1	108	78
65	4	510	123.2	...	....	...	....	...	...
Mean		487	111.5	200	113.3	231	107.7	280	201
SD		83	5.8	39.0	7.0	43	7.0	61	48
SE_M		± 10.8	± 0.76	± 5.3	± 0.98	± 5.8	± 0.95	± 8.1	± 6.3

individual values represent the average of duplicate determinations made on 80 specimens for 32 animals receiving the minimum amount of fat and 58 specimens for 13 animals receiving the control diet. Statistical analysis of these results was made and are summarized for the two groups in Table III. It will be noted that although there is considerable variation within each group, there is a statistically significant difference between the means of all the values for the two groups of animals. In every case the value of P is less than .0001. However, differences in the iodine number of the fatty acids in the serum are greater in proportion to their standard errors than those of the amount of fatty acid. Also, the mean iodine number of the acetone insoluble or phospholipid fatty acids of the fat deficient animals is higher than for the acetone soluble fatty acids which are derived from glycerides and cholesterol esters. In the control animals, on the other hand, the acetone soluble fatty acids are more unsaturated than the phospholipid fatty acids. Differences in the two groups of animals in the amount and iodine number of the fatty acids as well as the cholesterol and cholesterol ester content of the serum are illustrated graphically in Figures 1 to 4.

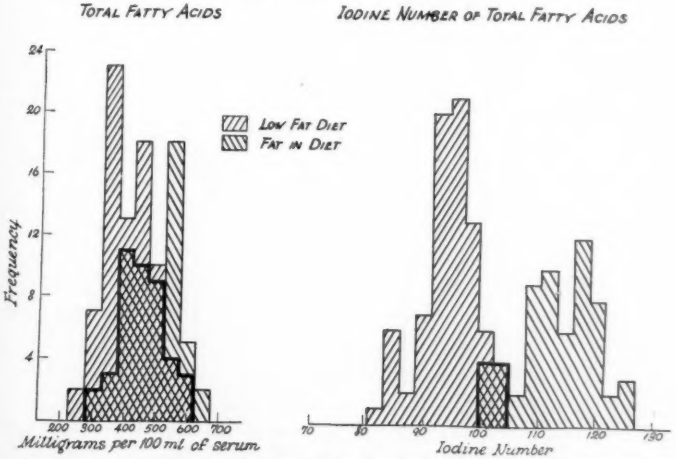

Fig. 1. Frequency Distribution of the Amount and Iodine Number of the Total Fatty Acids in Serum of Dogs on Low Fat Diet and With Fat in Diet (Lard).

TABLE III

Statistical Summary of Results of Tables I and II

Diet	Total Fatty Acids		Acet. Sol. F. A.		Acet. Insol. F. A.		Cholesterol	
	mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
Low fat (1% cal.)								
Mean + SE_M	412± 9.0	94.0±0.49	160±4.2	89.9±0.77	189±5.9	95.9±0.74	189±5.9	131±4.5
Lard (29% cal.)								
Mean + SE_M	487±10.8	111.5±0.76	200±5.4	113.3±0.98	230±5.8	107.7±0.95	280±8.1	201±6.4
x/θ	5.1	19	5.8	18	4.9	9.8	9.1	9.1
P	–.0001	–.0001	–.0001	–.0001	–.0001	–.0001	–.0001	–.0001

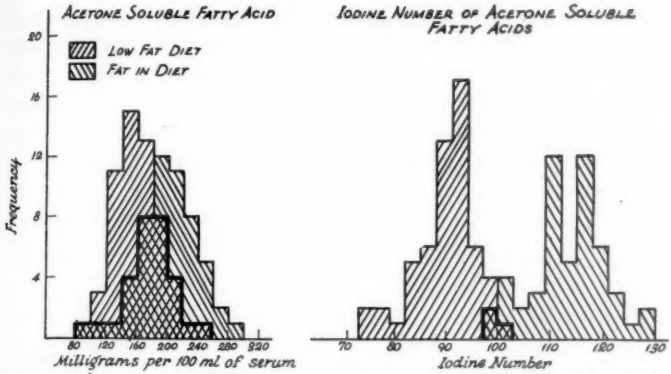

FIG. 2. Frequency Distribution of the Amount and Iodine Number of the Acetone Soluble Fatty Acids in Serum of Dogs on Low Fat Diet and With Fat in Diet (Lard).

The results of the separation of the fatty acids of the serum derived from the phospholipids, glycerides and cholesterol esters are presented in Table IV. Significant differences in the amount and iodine number of the phospholipid fatty acids are apparent as in Tables I and II. There is no difference in

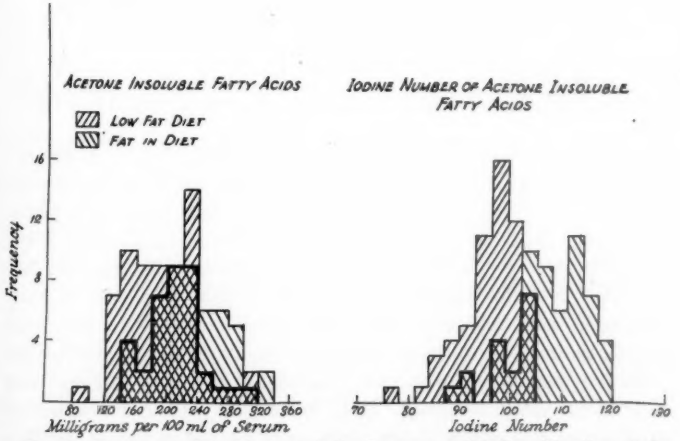

FIG. 3. Frequency Distribution of the Amount and Iodine Number of the Acetone Insoluble Fatty Acids in Serum of Dogs on Low Fat Diet and With Fat in Diet (Lard).

the amount or iodine number of the fatty acids combined as glycerides for the two groups of dogs. The greatest difference between the fatty acids of the serum of animals showing characteristic fat deficient skin changes and the control animals receiving 29 per cent calories as lard occurs in the iodine number of the fatty acids combined as cholesterol ester.

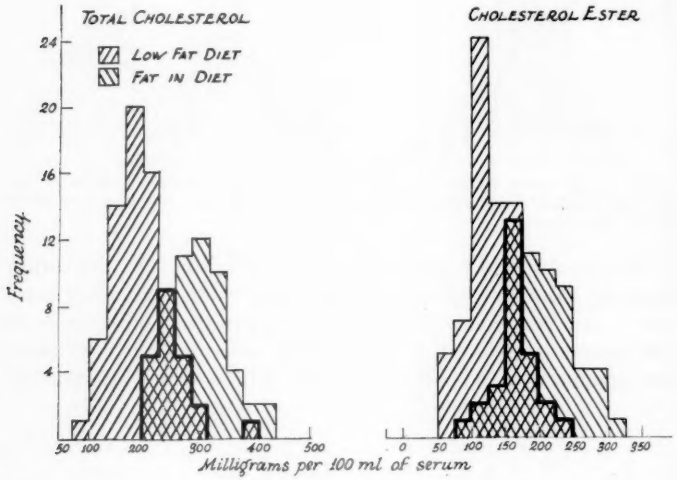

FIG. 4. Frequency Distribution of the Amount of Cholesterol and Cholesterol Ester in Serum of Dogs on Low Fat Diet and With Fat in Diet (Lard).

Addition of fat to diet: Results of the lipid analysis of the serum of dogs before and after the incorporation of fat in the diet are reported in Table V. It may be noted that the iodine number of the total fatty acids increased in some instances within a month after the incorporation of fat in the diet (dogs 26, 58, 61, 63). Visible improvement in the appearance of the skin and hair was observed about the same time. The rate and degree of improvement seemed to depend on the length of the depletion period, the severity of depletion and the amount and kind of fat which was fed. How these correlate with the iodine number of the serum fatty acids may be noted in the various dogs.

Dogs 46 and 47 were litter mates, each received 5 per cent of its calories as fat. At the end of 3 months dog 46, receiving lard, had a complete coat of new hair and there was no evidence of desquamation. The serum fatty acids showed an increase in iodine number from 91 to 99 during this time.

Dog 47, receiving butterfat, showed less improvement than dog 46 during the first three months. The ear canals had healed and there was growth of new hair, however, there was extensive desquamation over the entire body and there were

TABLE IV

Fatty Acid Fractions in Serum of Dogs

Dog	Total Fatty Acids mg%	Total Fatty Acids I.N.	Phospholipid Fatty Acids mg%	Phospholipid Fatty Acids I.N.	Glyceride Fatty Acids mg%	Glyceride Fatty Acids I.N.	Cholesterol Ester Fatty Acids mg%	Cholesterol Ester Fatty Acids I.N.
Low fat diet (Fat one per cent of calories)								
22	480	92.3	233	96.0	..	57.1	105	100.7
20	528	97.9	267	98.2	..	75.0	136	109.7
19	496	95.4	239	102.4	..	79.1	114	101.0
22	509	93.4	225	94.5	90	75.6	112	101.8
20	432	92.3	187	100.0	71	...	96	96.7
19	491	95.8	137	94.8	79	...	119	102.3
20	462	93.1	218	97.3	43	71.0	128	103.3
25	450	94.4	212	103.2	43	63.4	100	107.8
26	408	94.0	136	103.0	90	75.2	102	98.0
19	402	94.6	182	93.6	58	70.8	101	107.9
Mean	466	94.3	204	98.3	68	70.9	111	102.9
S.D.	40	2.5	41	3.6	19	6.8	12	3.3
SE_M	± 12	± 0.85	± 14	± 1.2	± 7	± 2.5	± 4	± 1.1
Lard in diet (29 per cent of calories)								
18	530	116.8	237	112.4	..	53.7	156	132.0
15	530	117.9	207	120.0	..	75.1	171	139.2
16	510	116.6	219	114.9	..	...	150	134.9
16	501	113.5	201	110.5	94	72.2	125	135.0
17	542	108.4	218	107.8	72	79.4	146	121.0
18	565	108.7	244	107.2	40	60.6	143	120.2
15	563	110.8	237	111.9	74	...	150	125.1
23	358	115.9	215	....	73	79.9	127	132.5
24	496	113.6	221	120.0	63	69.9	117	134.9
20	412	114.1	248	109.3	48	75.5	108	128.9
20	452	107.3	221	104.3	68	69.1	113	127.3
Mean	496	113.0	224	111.8	67	70.6	137	130.1
S.D.	62	3.6	14	5.6	15	8.0	19	5.6
SE_M	± 19	± 1.1	± 4	± 1.8	± 2	± 2.6	± 6	± 1.8
t.diff.	1.26	14.6	1.53	6.03	0.13	0.08	3.4	11.8
P	.25	< .01	.15	< .01	> .50	> .50	< .01	< .01

TABLE V

Serum Lipid Values and Gross Appearance of Animals Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F. A.		Acet. Insol. F. A.		Cholesterol		Gross Appearance
			mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%	
26	Low fat	10	416	91	145	83	181	98	209	142	Extensive desquamation
	Lard, 5% cal.	1	394	100	149	103	106	97	217	148	New soft skin, extensive peeling of old skin
	Lard, 5% cal.	3	486	107	205	108	224	107	275	202	Skin clear, no desquamation
46	Low fat	5	315	91	151	79	125	103	147	90	Severely deficient*
	Lard, 5% cal.	3	426	99	151	93	225	99	260	170	No desquamation, skin good new coat of hair
	Lard, 5% cal.	4	488	103	162	103	278	98	254	154	Hair oily
47	Low fat	5	349	94	129	92	171	96	178	120	Loss of hair, skin peeling
	Butterfat, 5% cal.	3	452	91	145	94	235	92	237	152	Ears improved, extensive desquamation, infected areas, new hair
43	Low fat	30	365	94	142	73	161	97	148	111	Severely deficient*
	Lard, 10% cal.	4	509	110	...	...	...	...	234	177	Greatly improved but partial alopecia
	Lard, 10% cal.	12	615	106	...	...	...	...	...	...	Hair coarse, partial alopecia

TABLE V—(Continued)

Serum Lipid Values and Gross Appearance of Animals Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F. A.		Acet. Insol. F. A.		Cholesterol		Gross Appearance
			mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%	
55	Low fat	15	428	92	...	...	...	...	154	131	Severely deficient*
	Lard, 15% cal.	2	500	112	...	...	...	...	...	...	Skin, ear, feet good, hair oily
	Lard, 15% cal.	5	600	122	...	...	...	...	...	...	Good except for dry hair
58	Low fat	3	237	85	...	...	...	...	86	48	Severely deficient*
	Lard, 29% cal.	1	334	100	155	100	147	101	117	106	Ears good, skin good, new oily hair
	Lard, 29% cal.	6	287	106	86	102	141	99	108	78	Skin, hair excellent
37	Low fat	31	364	92	120	84	143	94	136	106	Severely deficient*
	Baconfat, 29% cal.	7	310	104	127	108	154	102	163	112	Ears, feet, skin good, hair on back dry
	Baconfat, 29% cal.	34	360	108	117	112	172	103	177	144	Good condition
39	Low fat	32	400	98	160	98	223	95	231	162	Severely deficient*
	Butterfat, 29% cal.	3	429	100	208	77	182	95	243	150	Ears good, feet swollen, skin thickened, hair coarse
	Butterfat, 29% cal.	25	528	100	209	100	220	100	257	206	Good condition
53	Low fat	15	386	85	...	...	...	...	117	82	Severely deficient*
	Linoleate, 1%	2	396	103	...	...	...	...	...	...	Ears good, no peeling but skin and hair dry
	Linoleate, 1%	4	396	106	147	106	152	113	156	105	Good condition but back hair coarse

TABLE V—(Continued)

Serum Lipid Values and Gross Appearance of Animals Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. F. A.		Acet. Insol. F. A.		Cholesterol		Gross Appearance
			mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%	
61	Low fat	3	389	87	...	...	...	...	142	71	Severely deficient*
	Linoleate, 1%	1	352	92	160	90	116	96	117	101	Soft skin, new hair, feet swollen
	Linoleate, 1%	3	410	105	...	...	...	...	...	...	Greatly improved, feet good
	Linoleate, 1%	4	379	100	...	...	...	...	...	...	Loss of hair, dryness of skin
	Linoleate, 1% + 5% lard	1½	463	104	192	104	221	104	190	146	Desquamation, new hair, lessened redness
63	Low fat	3	333	91	...	...	...	...	138	72	Severely deficient*
	Arachidonate, 1%	1	368	101	151	92	135	112	117	...	One ear clear, fine desquamation, fine growth of hair, paws red
	Arachidonate, 1%	1½	372	104	160	91	190	106	162	106	Infected areas nearly healed, paws red, fine desquamation, new hair

*A severely deficient animal shows infected ears, extensive peeling of skin, loss of hair, reddened, edematous feet.

infected areas on the head. There was no change in the iodine number of the fatty acids during this interval.

Dog 43, receiving 10 per cent calories as lard, showed definite gross improvement in four months but complete and permanent cure did not take place in a period of 12 months. The iodine number rose, however, to that of the control animals.

Dog 55, receiving 15 per cent calories as lard, showed marked improvement in two months and remained in good condition except for dry hair and skin for five months. Coincident with this improvement, there was an increase in the degree of unsaturation of the serum fatty acids.

Dog 58, receiving 29 per cent calories as lard, showed marked improvement in one month, although the new hair was quite oily. By six months the dog was in excellent condition. There was a gradual rise in iodine number of the serum fatty acids.

Dog 37, receiving 29 per cent calories as baconfat, within two weeks, showed improvement of the feet and ears, particularly. No desquamation was evident after one month. The dog was in good condition in three months and the serum fatty acids showed more unsaturation than during the low fat period.

Dog 39, receiving 29 per cent calories as butterfat, showed a slow but progressive improvement. At the end of three months, the ears had healed but the feet were still swollen and red, the skin was greatly thickened at the joints and the hair was extremely coarse. The iodine number of the serum fatty acids changed very little.

Dog 53, receiving one per cent calories as methyl linoleate showed definite improvement in appearance in two months and continued to improve during a four month period. There was a rise in the iodine number of the serum fatty acids to the control level.

Dog 61, receiving one per cent calories as methyl linoleate, showed definite progressive improvement in three months but at the end of four months, desquamation was evident and loss of hair was quite pronounced. The iodine number of the fatty acids showed a slight drop during this period. When 5 per cent of the caloric intake as lard was given in addition to the methyl

TABLE VI

Serum Lipid Values of Dogs Receiving Fat in Diet, as Lard, Baconfat, Butterfat or Crisco

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. Fatty Acids		Acet. Insol. Fatty Acids		Cholesterol		Gross Appearance
			mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%	
52	Lard, 5% cal.	2	441	102.5	171	107.4	170	101.5	256	168	Extensive loss of hair
	Lard, 29% cal.	3	430	118.4	169	129.6	229	107.3	224	191	Skin soft, hair coarse
	Lard, 29% cal.	8	401	110.3	161	115.2	156	104.8	274	116	Slight dryness of skin and hair
48	Baconfat, 5% cal.	2	412	108.5	124	110.0	194	100.0	279	138	Loss of hair, desquamation
	Baconfat, 29% cal.	8	456	116.4	178	127.5	168	104.0	240	199	Skin and hair good
50	Butterfat, 5% cal.	3	508	92.4	218	81.0	245	94.0	264	182	Loss of hair, desquamation
	Butterfat, 29% cal.	4	565	96.8	213	96.2	256	98.7	267	227	Hair coarse, some desquamation
	Butterfat, 29% cal.	9	491	106.0	213	100.9	195	111.5	303	172	Dryness of skin & hair evident
49	Crisco, 5% cal.	2	462	95.3	170	95.9	165	95.6	260	170	Loss of hair, desquamation
	Crisco, 29% cal.	8	478	108.4	196	108.5	138	108.5	225	191	Slight dryness of skin and hair

linoleate, there was renewed growth of hair and lessened redness of the skin. Desquamation was still evident. The iodine number rose slightly.

Dog 63, receiving one per cent calories as ethyl arachidonate, showed gradual improvement during the six weeks observation period. The serum fatty acids became more unsaturated.

Further evidence of the correlation of the iodine number of the serum fatty acids and the gross appearance of the skin and hair is indicated by data in Table VI. Litter mate puppies given 5 per cent caloric fat as lard, baconfat, butterfat or crisco, respectively, all showed loss of hair and desquamation at this level of fat. When the fat was increased to 29 per cent of the calories, a rise in the iodine number resulted and improvement of the skin and hair was noted.

Lipids of Skin

Entire skin to facial layer: In Table VII are summarized the data for the lipid analysis of sections of skin taken from 11 newborn puppies, and 31 dogs receiving the following diets: 12, low fat diet; 12, 29 per cent lard; 2, 29 per cent baconfat; 2, 29 per cent butterfat; 1, 29 per cent crisco; 1, one per cent ethyl arachidonate; 1, one per cent methyl linoleate; 1, one per cent methyl linoleate plus 5 per cent caloric lard. The total fatty acid content was lowest in the newborn group. Differences in the amount of fatty acid are not significant in the other groups. It will be noted, however, that the iodine number of the total fatty acids for the skin of the newborn puppies and the control group receiving 29 per cent fat as lard are essentially the same, 72 and 70, respectively, whereas, the mean value for the low fat group is significantly lower, 58. The iodine values for the dogs receiving baconfat and crisco (dogs 37, 48, 49) were also in the same range as the control group. On the other hand, the skin of the animals receiving butterfat was definitely lower. Likewise the animals receiving one per cent fat as ethyl linoleate showed values between those of the control and low fat groups. Surprisingly enough, the skin of the dog receiving ethyl arachidonate showed an iodine number similar to those of the low fat group. It should be pointed out that grossly the skin of the dogs receiving the

esters of linoleic and arachidonic acid were not healthy in appearance as redness and desquamation were still evident and there was considerable loss of hair. With one exception, there is very little difference in the cholesterol and cholesterol ester values of the skin of animals maintained on the various diets. The ester content of the skin of newborn puppies is exceedingly small, an average of 0.08 per cent compared with a range of 0.23 to 0.33 for the older puppies. A graphic representation of the iodine number of the total fatty acids of the skin in relation to the length of time on each diet is presented in Figure 5.

TABLE VII

Lipid Analysis of Skin of Dogs

Dog	Diet	Duration months	Total Fatty Acids		Cholesterol	
			percent	I.N.	Total	Ester
28	Newborn		4.2	69.1	0.34	0.08
29	Newborn		1.0	70.2	0.42	...
30	Newborn		1.9	65.8	...	...
31	Newborn		1.6	77.5	0.37	0.09
34	Newborn		2.0	74.8	0.46	0.13
36	Newborn		2.6	69.4	0.36	0.08
..	Newborn		0.9	72.8	0.29	0.05
..	Newborn		0.9	73.8	0.36	0.05
..	Newborn		1.2	78.2	0.45	0.10
..	Newborn		1.3	71.0	0.28	0.06
..	Newborn		0.9	69.4	0.36	0.08
Mean			1.7	72.0	0.37	0.08
S.D.			0.9	3.6	0.18	0.01
SE_M			± 0.3	± 1.1	± .06	± 0.004
19	Low fat, 1% cal.	5	14.1	53.1	...	...
20	Low fat, 1% cal.	11	8.8	61.8	0.28	0.14
21	Low fat, 1% cal.	3	5.1	54.6	0.61	0.27
22	Low fat, 1% cal.	4	12.8	50.8	0.36	0.19
22	Low fat, 1% cal.	10	22.8	59.9	0.38	0.30
25	Low fat, 1% cal.	6	...	66.6	0.48	0.41
26	Low fat, 1% cal.	6	5.8	57.0	0.46	0.28
27	Low fat, 1% cal.	6	5.2	55.1	0.56	0.35
42	Low fat, 1% cal.	41	8.1	62.2	0.57	0.39
51	Low fat, 1% cal.	5	1.1	52.7	0.52	0.33
54	Low fat, 1% cal.	19	19.4	63.3	0.58	0.54
57	Low fat, 1% cal.	9	15.0	59.9	...	...
60	Low fat, 1% cal.	5	8.7	58.9	0.62	0.47
Mean			10.5	58.1	0.49	0.33
S.D.			6.0	4.5	0.12	0.11
SE_M			± 1.8	± 1.3	± 0.04	± 0.03
15	Lard, 29% cal.	11	5.0	67.7	0.20	0.14

TABLE VII—(Continued)

Lipid Analysis of Skin of Dogs

Dog	Diet	Duration months	Total Fatty Acids		Cholesterol	
			percent	I.N.	Total	Ester
15	Lard, 29% cal.	22	10.4	69.4	0.35	0.18
16	Lard, 29% cal.	5	18.2	65.2	0.34	0.17
17	Lard, 29% cal.	16	6.8	71.4	0.45	0.24
18	Lard, 29% cal.	16	12.1	73.8	0.49	0.27
18	Lard, 29% cal.	29	...	71.3	...	...
19	Lard, 29% cal.	18	...	76.4	0.54	...
20	Lard, 29% cal.	2	9.1	68.2	0.35	0.17
20	Lard, 29% cal.	11	17.2	68.2	0.33	0.30
23	Lard, 29% cal.	5	26.1	73.0	0.43	0.32
24	Lard, 29% cal.	6	6.3	69.1	0.43	0.26
24	Lard, 29% cal.	12	6.0	72.0	0.43	0.23
27	Lard, 29% cal.	4	6.3	66.8	0.47	...
27	Lard, 29% cal.	7	23.9	67.2	0.31	0.30
35	Lard, 29% cal.	52	25.1	76.8	...	0.18
52	Lard, 29% cal.	9	13.9	71.7	0.56	0.48
58	Lard, 29% cal.	6	29.5	66.1	0.47	...
Mean			14.4	70.2	0.41	0.25
S.D.			8.0	3.4	0.03	0.07
SE_M			± 2.1	± 0.8	± 0.008	± 0.01
37	Baconfat, 29% cal.	9	26.5	73.6	...	...
48	Baconfat, 29% cal.	9	10.0	68.5	0.32	0.23
Mean			18.2	71.0	0.32	0.23
49	Crisco, 29% cal.	9	25.8	78.2	...	0.31
50	Butterfat, 29% cal.	9	24.6	64.2	0.38	...
39	Butterfat, 29% cal.	25	20.3	62.4	0.38	...
Mean			22.4	63.3	0.38	...
61	Methyl linoleate, 1% cal. + Lard, 5% cal.	6 + 1½				
	thigh		5.3	64.6	0.41	0.28
	ventral		4.4	63.1	0.36	0.26
Mean			4.8	63.8	0.38	0.27
53	Methyl linoleate, 1% cal.	4½				
	thigh		13.5	64.2	0.46	0.36
	ventral		11.7	65.7	0.19	0.17
Mean			12.6	64.9	0.32	0.26
63	Ethyl arachidonate, 1% cal.	1½				
	thigh		4.0	55.0	0.43	0.25
	ventral		3.2	56.8	0.17	0.10
Mean			3.6	55.9	0.30	0.17

Desquamated skin: The skin of an animal which shows extensive peeling feels very greasy. In an attempt to study the composition of the desquamated epidermis, portions of it were peeled off gently with thumb forceps and extraction of the total lipids was made as for the skin as a whole. When fat is

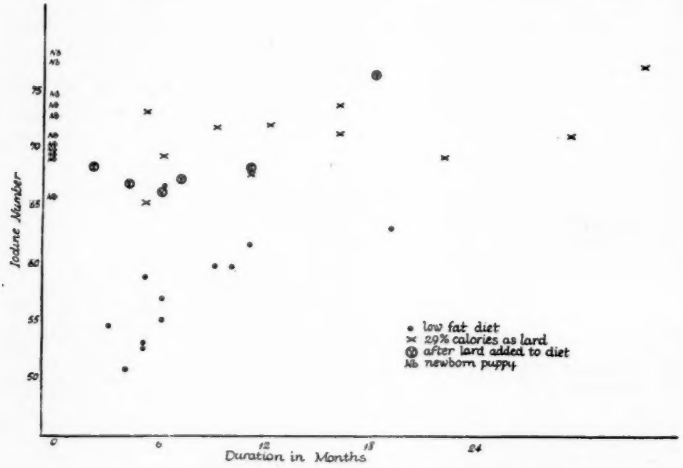

FIG. 5. Iodine Number of Total Fatty Acids of Dogs.

added to the diet of an animal which has been on the one per cent caloric fat intake, desquamation continues for some time while new healthy skin is being formed. Also it was found that a level of 5 per cent lard or butterfat was not sufficient to cure or prevent the development of desquamation. In Table VIII are recorded the analyses of the desquamated material for seven animals, three on the low fat diet, three receiving 5 per cent fat as lard or butterfat, and one receiving 29 per cent fat as lard after maintenance on the low fat diet. Three observations may be made readily from these data. First, the average values for the amount and iodine number of the total fatty acids for the desquamated skin are essentially the same regardless of the diet. Second, all specimens of desquamated skin show a high percentage of total cholesterol and cholesterol ester. Third, the iodine number of the total fatty acids is

TABLE VIII

Lipid Components of Desquamated Skin (Wet Basis)

Dog	Fat	Diet % Cal.	Duration months	Total Fatty Acids percent	Total Fatty Acids I.N.	Cholesterol Total percent	Cholesterol Ester percent
51	M.F.	1.0	4	10.2	29.1	5.95	4.48
51	M.F.	1.0	6	7.4	32.8	4.06	1.90
42	M.F.	1.0	33	16.0	12.8	8.88	6.92
43	M.F.	1.0	29	7.5	27.0	4.56	1.50
Avg.				10.3	25.4	5.36	3.70
52	Lard	5.0	2	10.0	28.1	5.57	4.91
52	Lard	30.0	2	15.0	20.6	8.57	5.88
46	Lard	5.0	6	13.1	23.3	6.50	4.99
46	Lard	5.0	7	15.9	29.3	7.34	6.31
47	B.F.	5.0	5	15.2	22.9	8.42	6.30
47	B.F.	5.0	6	7.7	33.1	3.36	1.10
19	Lard	30.0	2	8.0	22.5	5.30	2.90
Avg.				12.1	25.7	6.47	4.63

definitely lower than for the skin as a whole. When compared on the dry basis (Table IX) the desquamated portion of skin shows six or more times the amount of cholesterol and cholesterol ester found in the skin as a whole.

TABLE IX

Lipids of Whole Skin and Desquamated Skin (Dry Basis)

	Total Fatty Acids percent	Total Fatty Acids I.N.	Cholesterol Total percent	Cholesterol Ester percent
Low fat diet				
Whole Skin	20.8	58.1	1.30	0.75
Desq. Skin	11.3	25.4	5.90	4.07
Fat in diet				
Whole Skin	43.3	70.2	1.02	0.66
Desq. Skin	13.3	25.7	7.12	5.10

Subcutaneous Tissue

In Table X are reported the values for the amount and iodine number of the total fatty acids. The number of analyses are fewer than for the skin inasmuch as newborn puppies and severely emaciated fat deficient animals have very little adipose tissue in the subcutaneous layers. When present, the amount of fat in this tissue showed considerable variation but again

TABLE X

Total Fatty Acids of Subcutaneous Tissue

Dog	Diet	Duration on diet (months)	percent	I.N.
24	Newborn		1.8	71.4
30	Newborn		1.5	77.1
..	Newborn		9.4	69.8
..	Newborn		0.1	82.4
..	Newborn		0.1	84.7
	Mean		2.6	77.1
	S.D.		3.5	6.0
	SE_M		± 1.7	± 3.0
19	Low fat	5	59.4	48.8
20	Low fat	11	53.0	56.7
22	Low fat	10	67.7	58.4
25	Low fat	6	75.5	56.4
26	Low fat	6	2.3	45.0
27	Low fat	6	47.2	51.7
42	Low fat	41	62.2	61.9
59	Low fat	10	76.6	62.9
	Mean		55.5	55.2
	S.D.		21.6	6.2
	SE_M		± 8.2	± 2.3
15	Lard, 29% cal.	11	69.6	65.4
15	Lard, 29% cal.	22	80.0	73.3
16	Lard, 29% cal.	5	57.4	61.3
17	Lard, 29% cal.	16	80.2	72.7
18	Lard, 29% cal.	16	79.7	72.9
18	Lard, 29% cal.	29	78.0	73.8
19	Lard, 29% cal.	18	79.3	71.2
20	Lard, 29% cal.	2	69.5	66.4
20	Lard, 29% cal.	11	76.0	65.6
23	Lard, 29% cal.	3	62.8	67.3
24	Lard, 29% cal.	6	4.2	65.2
24	Lard, 29% cal.	12	49.7	63.7
27	Lard, 29% cal.	4	52.8	65.2
27	Lard, 29% cal.	7	81.7	67.0
35	Lard, 29% cal.	52	45.7	70.8
52	Lard, 29% cal.	9	79.1	68.4
	Mean		65.4	68.1
	S.D.		19.7	3.7
	SE_M		± 5.1	± 0.9
37	Baconfat, 29% cal.	9	63.9	71.5
48	Baconfat, 29% cal.	9	90.0	68.5
	Mean		76.9	70.0

TABLE X—(Continued)

Total Fatty Acids of Subcutaneous Tissue

Dog	Diet	Duration on diet (months)	percent	I.N.
39	Butterfat, 29% cal.	25	79.6	48.9
50	Butterfat, 29% cal.	9	86.1	54.6
	Mean		82.8	51.8
49	Crisco, 29% cal.	9	83.4	78.9
63	Arachidonate, 1% cal.	1½	68.8	54.5

the iodine number of the total fatty acids followed closely that of the skin. Data for the skin and subcutaneous tissues are summarized statistically in Table XI.

TABLE XI

Statistical Summary of Results for Skin and Subcutaneous Tissue (Tables VII & X)

Fraction	Tissue	Differences between means	t	P
Total Fatty Acids				
Lard (29%)—low fat	skin	3.8	1.3	.20
Lard (29%)—newborn	skin	12.7	5.0	< .01
Low fat—newborn	skin	8.9	4.6	< .01
I.N. of T.F.A.				
Lard (29%)—low fat	skin	12.1	8.1	< .01
Lard (29%)—newborn	skin	−1.8	1.3	.22
Low fat—newborn	skin	−13.9	7.9	< .01
Total Cholesterol				
Lard (29%)—low fat	skin	−.08	2.0	.05
Lard (29%)—newborn	skin	.04	1.2	.23
Low fat—newborn	skin	.12	3.1	< .01
Cholesterol Ester				
Lard (29%)—low fat	skin	−.08	2.0	.05
Lard (29%)—newborn	skin	.17	5.7	< .01
Low fat—newborn	skin	.25	6.2	< .01
Total Fatty Acids				
Lard (29%)—low fat	subcutaneous	9.9	1.0	.03
Lard (29%)—newborn	subcutaneous	62.8	6.7	< .01
Low fat—newborn	subcutaneous	51.9	5.2	< .01
I.N. of T.F.A.				
Lard (29%)—low fat	subcutaneous	12.9	6.1	< .01
Lard (29%)—newborn	subcutaneous	−8.9	3.7	< .01
Low fat—newborn	subcutaneous	−21.8	18	< .01

Liver, Kidney, Heart

From the analyses reported in Table XII, it will be noted that with or without fat in the diet, chemically, there was no evidence of fatty livers. The amount of fat present was very nearly the same regardless of the dietary regimen. On the other hand, the unsaturation of the fatty acids was definitely decreased when fat was lacking in the diet. The differences were reflected both in the acetone soluble and acetone insoluble fractions. Most of the acetone soluble fatty acids may be considered to be derived from glycerides because of the low cholesterol ester content of the liver. The phospholipid fatty acids were uniformly more unsaturated than those of the glycerides. The results of the analysis of the kidneys of animals on the various diets indicate that in comparable groups, the values do not differ greatly from those of the liver. Fewer analyses were made on the heart than for the other tissues but again the trend is the same. In addition, however, the phospholipid fatty acids of the heart had iodine numbers considerably greater than for any other tissue.

Discussion

It was shown many years ago that the nature of dietary fat affected the composition of the depot fat (Henriques and Hansen, 1901–1902, Ellis and Isbell, 1926, Anderson and Mendel, 1928). Many similar studies have been carried out. However, the influence of diet on blood fats, particularly the nature of the fatty acids, has not been studied extensively. Our findings regarding the iodine number of the serum fatty acids of dogs receiving various amounts and kinds of fat clearly indicate that the blood fats not only reflect the nature of the dietary fat but also correlate well with the appearance of the skin. In the correlation of the iodine number of the serum fatty acids and the nature of dietary fat, higher iodine numbers were observed when lard, baconfat or crisco were fed than when butterfat was given. The effectiveness of these fats in curing the deficiency syndrome was of this same order. The total amount of fatty acid in the serum of animals maintained on a low fat diet clearly shows that these animals synthesize

TABLE XII

Lipid Composition of Liver, Kidney and Heart of Dogs

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. Fatty Acids		Acet. Insol. Fatty Acids		Cholesterol	
			percent	I.N.	percent	I.N.	percent	I.N.	Total percent	Ester percent
LIVER										
51	Low fat, 1% cal.	5	2.4	91.0	1.3	80.0	0.4	107.8	0.29	0.07
59	Low fat, 1% cal.	10	2.2	92.9	0.4	64.3	0.3	109.5	0.29	0.15
22	Low fat, 1% cal.	10	2.7	83.5	0.4	72.2	0.8	96.9	0.40	0.10
54	Low fat, 1% cal.	19	2.4	91.6	0.6	77.7	0.5	100.7	0.41	0.27
42	Low fat, 1% cal.	4	3.3	82.9	1.5	76.5	1.7	97.2	0.61	...
		Mean	2.6	88.2	0.8	74.1	0.7	102.4	0.40	0.15
19	Lard, 29% cal.	2	1.9	95.7	0.6	68.5	0.6	122.8	0.29	0.10
20	Lard, 29% cal.	2	2.3	110.5	0.4	77.3	1.4	115.0	0.68	0.14
58	Lard, 29% cal.	6	2.2	113.5	0.5	80.5	1.0	121.9	0.29	0.07
27	Lard, 29% cal.	7	2.3	110.0	..	...	1.4	115.2	0.69	0.11
52	Lard, 29% cal.	9	2.1	114.3	0.9	99.6	1.1	121.8	0.32	...
15	Lard, 29% cal.	22	2.3	109.5	0.6	82.6	1.4	123.4	0.33	0.07
18	Lard, 29% cal.	27	2.3	119.8	0.4	72.9	1.6	119.6	0.84	0.10
35	Lard, 29% cal.	52	3.9	110.4	1.5	78.7	2.1	115.9	0.55	...
		Mean	2.4	110.5	0.7	80.0	1.3	119.4	0.50	0.10
37	Baconfat, 29% cal.	9	3.1	98.1	1.0	70.9	1.1	127.5	0.47	...
48	Baconfat, 29% cal.	9	2.0	119.5	0.4	75.1	1.3	127.3	0.26	0.16
		Mean	2.5	108.8	0.7	73.0	1.2	127.4	0.36	0.16
50	Butterfat, 29% cal.	9	2.1	111.6	0.4	70.4	1.4	113.3	0.34	0.19
39	Butterfat, 29% cal.	25	2.7	92.0	1.0	63.0	1.4	115.1	0.66	0.49
		Mean	2.4	101.8	0.7	66.7	1.4	114.2	0.50	0.34

TABLE XII—(Continued)

Lipid Composition of Liver, Kidney and Heart of Dogs

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. Fatty Acids		Acet. Insol. Fatty Acids		Cholesterol	
			percent	I.N.	percent	I.N.	percent	I.N.	Total percent	Ester percent
49	Crisco, 29% cal.	9	2.1	106.1	0.6	93.7	0.8	119.4	0.33	0.19
63	Ethyl arachidonate, 1% cal.	1½	2.1	100.1	0.4	77.1	1.2	138.1	0.14	0.03
61	Methyl linoleate, 1% cal. + Lard, 5% cal.	6 1½	2.5	110.4	0.6	79.3	0.8	117.1	0.29	0.14
53	Methyl linoleate, 1% cal.	4½	2.5	107.5	0.5	84.3	0.5	123.8	0.34	0.17
KIDNEY										
51	Low fat, 1% cal.	5	1.6	106.0	0.2	89.2	2.0	118.6	0.39	0.01
59	Low fat, 1% cal.	10	1.6	97.3	0.3	74.4	0.8	104.3	0.14	....
54	Low fat, 1% cal.	19	3.6	97.7	1.9	98.3	1.0	96.7	0.36	0.10
42	Low fat, 1% cal.	41	3.2	94.6	1.7	83.7	0.8	116.0	0.34	0.13
		Mean	2.5	98.9	1.0	86.4	0.9	108.9	0.32	0.08
19	Lard, 29% cal.	2	3.0	94.2	1.9	87.8	0.6	106.0	0.31	0.07
20	Lard, 29% cal.	2	2.1	116.4	0.9	110.6	1.1	119.7	0.53	0.04
38	Lard, 29% cal.	6	2.6	102.6	1.2	83.0	0.9	128.1	0.31	0.06
27	Lard, 29% cal.	7	2.1	110.5	0.7	95.0	1.3	123.4	0.63	...
52	Lard, 29% cal.	9	1.6	122.0	0.2	100.0	1.2	120.0	0.21	0.08
18	Lard, 29% cal.	27	2.4	95.6	1.0	78.0	1.3	106.9	0.71	0.42
35	Lard, 29% cal.	52	2.9	106.4	1.2	104.5	1.2	135.5	0.56	0.37
		Mean	2.4	106.8	1.0	94.1	1.1	119.9	0.46	0.16
37	Baconfat, 29% cal.	9	1.7	112.5	0.3	72.8	0.9	123.8	0.33	0.06
48	Baconfat, 29% cal.	9	2.4	101.8	1.3	90.8	0.9	123.9	0.35	0.21
		Mean	2.0	107.2	0.8	81.8	0.9	123.8	0.34	0.13

TABLE XII—(Continued)

Lipid Composition of Liver, Kidney and Heart of Dogs

Dog	Diet	Duration of diet (months)	Total Fatty Acids		Acet. Sol. Fatty Acids		Acet. Insol. Fatty Acids		Cholesterol	
			percent	I.N.	percent	I.N.	percent	I.N.	Total percent	Ester percent
39	Butterfat, 29% cal.	25	2.9	105.2	1.5	95.5	0.8	112.8	0.40	0.11
50	Butterfat, 29% cal.	9	2.0	115.6	0.6	98.5	1.3	118.8	0.40	0.17
		Mean	2.5	110.4	1.0	97.0	1.0	115.8	0.40	0.14
49	Crisco, 29% cal.	9	2.3	103.9	1.1	92.9	1.0	119.4	0.38	0.15
63	Ethyl arachidonate, 1% cal.	1½	1.7	108.4	0.2	84.9	1.3	115.0	0.49	0.06
61	Methyl linoleate, 1% cal. + Lard, 5% cal.	6 1½	2.3	115.0	0.8	84.6	1.3	119.3	0.40	0.07
53	Methyl linoleate, 1% cal.	4½	2.0	114.6	0.2	60.8	1.3	125.6	0.39	0.04
HEART										
51	Low fat	5	1.1	115.9	0.1	99.9	0.8	114.1	0.16	0.10
59	Low fat	10	1.4	114.6	0.5	74.2	0.7	110.5	0.07	0.02
54	Low fat	19	1.4	107.9	0.1	63.3	1.0	110.7	0.13	0.04
		Mean	1.3	112.8	0.2	79.1	0.8	111.8	0.12	0.08
19	Lard, 29% cal.	2	1.6	108.5	0.7	75.4	0.7	137.1	0.10	0.05
58	Lard, 29% cal.	6	1.6	128.9	0.4	76.9	0.7	135.1	0.13	0.05
35	Lard, 29% cal.	52	1.8	118.9	0.6	80.2	0.7	140.1	0.16	0.05
		Mean	1.7	118.8	0.6	77.5	0.7	137.4	0.13	0.05
37	Baconfat, 29% cal.	9	1.6	117.0	0.6	79.9	0.8	139.4	0.14	0.02
39	Butterfat, 29% cal.	25	1.7	108.1	0.6	63.1	0.5	125.9	0.13	0.05
63	Ethyl arachidonate, 1% cal.	1½	1.8	98.9	0.9	62.7	0.8	138.1	0.14	0.03
61	Methyl linoleate, 1% cal. + Lard, 5% cal.	6 1½	1.7	125.2	0.4	78.9	1.1	130.9	0.14	0.01
53	Methyl linoleate, 1% cal.	4½	1.4	126.6	0.4	75.1	1.1	139.2	0.12	0.01

fatty acids to maintain a high blood level. Particularly significant is the low degree of unsaturation of these fatty acids compared with those of control animals having fat in the diet and exhibiting a healthy skin.

During the recovery phase from the deficiency state when fat is incorporated in the diet, there is good correlation between the iodine number of the serum fatty acids and the gross appearance of the skin. From Table IV it is apparent that the cholesterol ester fatty acids are the most highly unsaturated fatty acids in the serum and this fraction shows the greatest difference in unsaturation between the fat free and the control group of animals. As shown by Miller and Hansen (1944) there is more cholesterol ester in the plasma than in the erythrocytes of blood, hence analysis of plasma or serum for unsaturated fatty acids will show greater differences between a healthy and abnormal state than the analysis of whole blood.

Definite as the relationship between dietary fat and the gross appearance of the skin seems to be, the skin lipids reflect this correlation less than the serum lipids. There is, however, a significantly lower range for the iodine number of abnormal fat deficient skin than for the skin of healthy control animals. The iodine number of the fatty acids of the skin of the animals receiving linoleic or arachidonic acid was between those of the fat free and control group. These animals were not completely cured of the deficiency. Interestingly enough, the skin of healthy newborn puppies showed a composition similar to that of mature control animals. The cholesterol ester fatty acids in the serum reflected the greatest differences in dietary fat but the very small amount of ester in the skin made it impossible to determine whether or not there was a qualitative difference in these fatty acids. The desquamated portion of skin from fat deficient puppies showed a high percentage of cholesterol and cholesterol ester but the fatty acids derived from this portion of skin had a very low iodine number. The amount of phospholipid was too small to evaluate its significance in the skin.

The phospholipid fatty acids of the liver, kidney and heart showed definite differences between the fat free and control group. The most striking finding was the very high iodine

number of the phospholipid fatty acids of the heart when lard, baconfat, arachidonic acid or linoleic acid were fed. With one exception all the values fell within a comparatively narrow range 137–141 compared with 126 for the butterfat group and 112 for the low fat group. These data showing the deposition of unsaturated fatty acids in the phospholipid fraction of tissues, particularly of the heart, are in confirmation of the findings of Rieckehoff, Holman and Burr (1949) and Widmer and Holman (1950) for rats.

Summary and Conclusions

Analyses of tissues of 42 dogs show there are significant differences in the lipid composition of the blood serum, skin, subcutaneous fat, liver, kidney and heart of animals maintained on regimens with and without fat in the diet.

The amount of cholesterol, cholesterol ester and total fatty acids as well as the iodine number of the fatty acids are lower in the serum of dogs reared on a diet containing one per cent of the calories as fat than in animals receiving 30 per cent of the calories as fat. Fractionation of the total fatty acids shows this to be true for the fatty acids derived from the phospholipids and the cholesterol esters but not for those derived from the glycerides. Highly significant differences were found in the degree of unsaturation of the phospholipid and cholesterol ester fatty acids between the two groups of animals. The most unsaturated fatty acids of the serum are found in the cholesterol ester fraction. Direct correlations were observed between the gross appearance of the skin and hair of the animals, the iodine number of the serum fatty acids, and the kind of dietary fat.

The skin and subcutaneous tissue of dogs on the various diets likewise showed the fatty acids to be less unsaturated in the animals showing fat deficiency symptoms than in those exhibiting a healthy skin. Although significantly different, the magnitude of these differences was less than in the serum of the same animals. Desquamated portions of the skin of fat deficient animals contain a high percentage of cholesterol and cholesterol ester.

The liver, kidney and heart of dogs maintained on diets with and without fat, did not show significant differences in the amount of fatty acid, cholesterol or cholesterol ester. The

iodine number of the fatty acids, however, varied with the kind of dietary fat. The phospholipid fatty acids of the heart were the most highly unsaturated fatty acids in these tissues.

The iodine numbers of the serum fatty acids, particularly those combined as cholesterol ester, reflect the composition of the dietary fat to a greater degree than the lipids of the skin, subcutaneous fat, liver, kidney, or heart.

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FAT IN THE DIET IN RELATION TO NUTRITION OF THE DOG

III. SPECTRAL ANALYSIS FOR UNSATURATED FATTY ACID CONTENT OF TISSUES FROM ANIMALS FED DIETS WITH AND WITHOUT FAT*

HILDA F. WIESE AND ARILD E. HANSEN

The desirability of ascertaining the distribution of the unsaturated fatty acids of serum and tissues of dogs maintained on various fat regimens became apparent with the accumulation of data which indicated correlations between dietary fat and the nutritional condition of the animals as well as between each of these factors and the iodine number of the tissue lipids. The iodine number of the total fatty acids may reflect unsaturation due to oleic acid and the more highly unsaturated fatty acids in addition to the ratio of saturated to unsaturated fatty acids. It was felt therefore that spectral analyses might help to show whether or not there was a preponderance of any specific unsaturated fatty acid group in animals receiving fat which did not appear in animals on a low fat regimen.

Material and Methods

Tissues of dogs maintained under the conditions previously described (Hansen and Wiese, 1951) were used for spectrographic study. Blood serum, skin, liver, kidney and heart of ten dogs were examined. The amount of material necessary for the absorption studies greatly exceeded that for the determination of total fatty acids and iodine number, hence, spectral analyses were made only on autopsy specimens. The total lipids were extracted from the tissues and the fatty acids precipitated as for the iodine number determinations. The alkali conjugated fatty acid soaps were prepared according to the method of Holman and Burr (1948) by heating about 100 mg. samples in 23

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per cent KOH-ethylene glycol reagent at 180° C for eight minutes. Spectral densities were read in methyl alcohol solution on a Beckman D. U. spectrophotometer at wave lengths of maximum absorption for 2, 3, 4, 5 and 6 double bond fatty acids. Average absorption coefficient values were calculated from duplicate samples.

Results

Absorption coefficients at various wave lengths for the alkali conjugated fatty acids of the serum, skin, liver, kidney and heart of animals maintained on regimens with and without fat are given in Table I.

Blood serum: It will be noted that at a wave length of 2350Å, which represents absorption due principally to dienoic acids with some contribution from 3, 4, 5 and 6 double bond acids, the values for $E^{1\%}_{1cm}$ are lowest when fat was lacking in the diet and are highest when lard, baconfat or methyl linoleate were fed. Intermediate values were found for butterfat. At this wave length, the $E^{1\%}_{1cm}$ value of the serum fatty acids of the animal receiving one per cent of its calories as ethyl arachidonate did not differ greatly from that of the low fat group. At 2700Å, where the three double bond fatty acids have their maximum absorption, there does not appear to be any orderly difference in the $E^{1\%}_{1cm}$ values. At 3000Å, however, where the maximum absorption of arachidonic acid occurs, the values for all the dogs receiving fat in the diet are considerably higher than for those of the low fat group. The results of the absorptions at 3475Å and 3750Å are variable. With this method of alkali conjugation they indicate the possible presence of 5 or 6 double bond fatty acids.

Skin: The fatty acids of the skin show the same striking trend at 2350Å as those of the serum, although the numerical values are much less than for the serum. At 3000Å the absorption coefficients are not as consistent as for the serum. At 3475Å and 3750Å the values are variable.

Liver: With one exception (dog 63) the same consistent trend in $E^{1\%}_{1cm}$ values is observed at 2350Å and 3000Å as was noted for the fatty acids of the serum. In the liver of the animal receiving ethyl arachidonate, the fatty acids show the same

TABLE I

TABLE I

Absorption Coefficients of Total Fatty Acids of Serum, Skin, Liver, Kidney and Heart of Dogs Maintained on Various Fat Regimens

Dog	Diet	Tissue	I.N.	$E^{1\%}_{1\ cm}$ 2350Å	$E^{1\%}_{1\ cm}$ 2700Å	$E^{1\%}_{1\ cm}$ 3000Å	$E^{1\%}_{1\ cm}$ 3475Å	$E^{1\%}_{1\ cm}$ 3750Å
54	Low fat, 1% cal.	Serum	93	133	124	21.3	7.2	3.5
59	Low fat, 1% cal.	Serum	96	143	135	17.5	5.2	2.4
19	Lard, 29% cal.	Serum	102	233	107	56.9	3.9	0.0
35	Lard, 29% cal.	Serum	116	268	163	83.5	7.2	1.0
58	Lard, 29% cal.	Serum	108	214	121	63.4	9.7	3.4
37	Baconfat, 29% cal.	Serum	108	296	131	69.5	8.6	1.5
39	Butterfat, 29% cal.	Serum	100	188	122	45.8	10.1	0.7
63	Ethyl arachidonate, 1% cal.	Serum	102	152	146	57.0	16.5	3.7
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Serum	112	222	122	64.3	7.7	2.5
53	Methyl linoleate, 1% cal.	Serum	110	241	131	59.3	6.4	2.5
54	Low fat, 1% cal.	Skin	61	8.5	5.5	3.0	1.6	0.9
57	Low fat, 1% cal.	Skin	61	14.9	10.7	6.0	3.3	1.9
59	Low fat, 1% cal.	Skin	67	18.5	12.1	7.5	4.1	2.7
60	Low fat, 1% cal.	Skin	59	17.8	12.8	5.3	3.5	2.2
19	Lard, 29% cal.	Skin	76	50.7	12.1	7.9	4.3	2.7
35	Lard, 29% cal.	Skin	77	41.8	7.1	4.5	2.2	1.2
58	Lard, 29% cal.	Skin	66	39.2	10.7	7.7	2.9	1.6
37	Baconfat, 29% cal.	Skin	74	61.0	10.9	7.1	3.3	1.9
39	Butterfat, 29% cal.	Skin	62	29.5	12.1	7.4	3.7	2.1
63	Ethyl arachidonate, 1% cal.	Skin	58	17.6	13.1	7.1	3.1	1.3
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Skin	65	46.2	16.3	11.1	4.8	2.9
53	Methyl linoleate, 1% cal.	Skin	64	37.1	12.1	8.1	3.4	2.0
54	Low fat, 1% cal.	Liver	92	102	88	16.5	5.7	3.8
59	Low fat, 1% cal.	Liver	93	115	101	14.1	5.2	2.4
19	Lard, 29% cal.	Liver	111	149	103	56.0	9.4	2.7
35	Lard, 29% cal.	Liver	114	165	129	82.6	14.2	5.6
58	Lard, 29% cal.	Liver	112	166	149	59.9	9.0	3.0
37	Baconfat, 29% cal.	Liver	98	150	102	57.9	12.0	4.3

TABLE I—(Continued)

Absorption Coefficients of Total Fatty Acids of Serum, Skin, Liver, Kidney and Heart of Dogs Maintained on Various Fat Regimens

Dog	Diet	Tissue	I.N.	$E^{1\%}_{1\ cm}$ 2350Å	$E^{1\%}_{1\ cm}$ 2700Å	$E^{1\%}_{1\ cm}$ 3000Å	$E^{1\%}_{1\ cm}$ 3475Å	$E^{1\%}_{1\ cm}$ 3750Å
39	Butterfat, 29% cal.	Liver	85	108	92	45.9	14.7	5.0
63	Ethyl arachidonate, 1% cal.	Liver	102	151	152	55.0	16.2	3.8
53	Methyl linoleate, 1% cal.	Liver	108	147	124	51.3	9.5	4.4
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Liver	111	162	127	57.1	5.7	1.3
54	Low fat, 1% cal.	Kidney	94	83	66	10.4	1.6	0.5
59	Low fat, 1% cal.	Kidney	96	127	111	19.7	2.0	0.2
19	Lard, 29% cal.	Kidney	94	145	107	61.1	4.5	1.2
35	Lard, 29% cal.	Kidney	106	156	115	70.1	7.0	1.4
58	Lard, 29% cal.	Kidney	103	135	108	52.9	3.3	0.1
37	Baconfat, 29% cal.	Kidney	107	159	129	69.3	7.6	2.5
39	Butterfat, 29% cal.	Kidney	101	125	94	52.0	9.7	2.0
63	Ethyl arachidonate, 1% cal.	Kidney	109	109	143	58.9	10.4	0.9
53	Methyl linoleate, 1% cal.	Kidney	115	179	153	75.3	3.3	0.4
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Kidney	113	161	122	63.0	2.3	0.2
54	Low fat, 1% cal.	Heart	99	129	98	17.5	3.9	1.2
59	Low fat, 1% cal.	Heart	110	160	134	22.0	5.0	1.8
19	Lard, 29% cal.	Heart	105	136	82	46.8	4.6	1.6
35	Lard, 29% cal.	Heart	97*	132	79	48.3	7.4	1.7
58	Lard, 29% cal.	Heart	130	256	151	91.8	7.1	1.8
37	Baconfat, 29% cal.	Heart	75*	109	45	29.5	8.9	2.9
39	Butterfat, 29% cal.	Heart	73*	73	48	27.0	7.8	2.0
63	Ethyl arachidonate, 1% cal.	Heart	96	114	93	35.0	10.4	4.9
53	Methyl linoleate, 1% cal.	Heart	109*	179	98	52.9	7.0	4.0
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Heart	126	247	142	82.2	9.1	4.5

*Original I.N.
Dog 35—119
Dog 37—117
Dog 39—102
Dog 53—126

values as for lard, baconfat, and linoleate. Again, when butterfat was the source of dietary fat, the fatty acids did not show the same relative amounts of 2 and 4 double bond fatty acids as when other fats were fed. At 2350Å the numerical values of the liver were consistently lower than for the serum but at 3000Å, they were very nearly the same.

Kidney: Less uniform trends appear in the absorption coefficients of the fatty acids of the kidney than for the serum and liver. However, when lard, baconfat or methyl linoleate were fed higher values were found at 2350Å and 3000Å than when the low fat diet was given.

Heart: Fewer results on heart are available for comparison inasmuch as oxidation of the fatty acids of this tissue occurred during storage of some of the specimens. This was indicated by a drop in the iodine number of the fatty acids after storage of the tissue. It seems reasonable to assume that the $E^{1\%}_{1cm}$ values in dogs 35, 37, 39 and 53 would have been considerably greater in the original tissue that the observed values show. In this event, the total fatty acids of the heart of all the dogs with the exception of 19 (receiving lard) and 63 (receiving ethyl arachidonate) appear to be more unsaturated than those of the liver and kidney. The heart was the only tissue which showed deterioration on storage.

Discussion

From the data presented correlations are indicated between the amount of specific unsaturated fatty acids of tissues and the kind of dietary fat fed. Although the data are too meagre for statistical interpretation, definite trends were observed in the amount of diene and tetraene fatty acids in the serum, skin, liver, kidney and heart of animals receiving fat containing linoleic and/or arachidonic acid. These animals exhibited either a healthy skin or one showing improvement from the fat deficient state. The absorption data at wave lengths of 2700Å, 3475Å and 3750Å also indicate the presence of 3, 5 and 6 double bond fatty acids. However, the values at these wave lengths were not consistent for any dietary regimen and their significance is not apparent at the present time. It is possible that the method of conjugation with 23 per cent KOH-ethylene glycol produced

Dog 39—102
Dog 53—126

some effects other than conjugation to account for absorption at these wave lengths. When pure linoleic acid is heated at 180° C for 8 minutes with the 23 per cent KOH-ethylene glycol reagent some absorption occurs at 2700Å, 3000Å, 3475Å and 3750Å. However, considering the probable concentration of linoleic acid in animal fats, the contribution of absorption at the longer wave lengths was considered to be negligible in comparison with the absorption at 2350Å.

Calculations for the exact percentage of 2, 3, 4, 5 and 6 double bond fatty acids in the tissues cannot be made until standards for all the polyene fatty acids in pure form are available. However, an estimate of the relative distribution of these fatty acids can be made. Extinction coefficients were determined for linoleic acid which was obtained from the Hormel Institute, Austin, Minnesota and for arachidonic acid which was kindly furnished by Doctor J. B. Brown, Columbus, Ohio. Extinction coefficients of Widmer and Holman (1950) were used for conjugated 5 and 6 double bond fatty acids. These absorption coefficients determined by conjugation with 23 per cent KOH-ethylene glycol reagent are as follows:

	$E^{1\%}_{1\ cm}$ of Conjugated Fatty Acid Soap				
	2350Å	2700Å	3000Å	3475Å	3750Å
Diene (linoleic)	846	...	...	...	...
Triene (linolenic)	550	698	...	...	...
Tetraene (arachidonic)	520	577	726	...	...
Pentaene	490	530	470	418	...
Hexaene	490	530	470	418	157

For comparative purposes estimations of the distribution of unsaturated fatty acids as per cent of the total fatty acids in the tissues have been made and are presented in Table II. With few exceptions the same trend observed in the $E^{1\%}_{1cm}$ values may be noted. More diene fatty acid is indicated in the serum, skin, liver and heart of animals receiving lard, baconfat or linoleic acid ester in the diet than in those on a low fat diet or one containing butterfat or the ester of arachidonic acid. The serum, liver and kidney of all the dogs receiving some form of fat show more tetraene fatty acid than the low fat group. With one exception (dog 63) this was also true for the fatty acids of heart. The inconsistent but high percentages of triene fatty acid in all the tissues except skin are difficult to explain at the present time.

TABLE II

Estimated Unsaturated Fatty Acid Distribution in Per Cent of Total Fatty Acids for Serum, Skin, Liver, Kidney and Heart

Dog	Diet	Tissue	2350Å diene	2700Å triene	3000Å tetraene	3475Å pentaene	3750Å hexaene
54	Low fat, 1% cal.	Serum	4.1	14.5	1.5	0.0	2.2
59	Low fat, 1% cal.	Serum	4.1	17.0	1.3	0.0	1.5
19	Lard, 29% cal.	Serum	15.7	8.7	7.2	0.9	0.0
35	Lard, 29% cal.	Serum	15.6	13.4	10.4	1.1	0.6
58	Lard, 29% cal.	Serum	13.3	9.6	7.2	0.2	2.1
37	Baconfat, 29% cal.	Serum	21.4	10.2	8.4	1.1	1.0
39	Butterfat, 29% cal.	Serum	10.3	11.7	4.7	1.9	0.5
63	Ethyl arachidonate, 1% cal.	Serum	4.1	12.8	5.3	1.6	2.3
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Serum	14.2	9.7	7.7	0.2	1.6
53	Methyl linoleate, 1% cal.	Serum	15.6	11.7	7.1	0.0	1.6
54	Low fat, 1% cal.	Skin	0.5	0.3	0.0	0.0	0.6
57	Low fat, 1% cal.	Skin	0.6	0.5	0.0	0.0	1.3
59	Low fat, 1% cal.	Skin	0.9	0.4	0.0	0.0	1.7
60	Low fat, 1% cal.	Skin	0.8	0.7	0.0	0.0	1.3
19	Lard, 29% cal.	Skin	4.6	0.3	0.0	0.0	1.7
35	Lard, 29% cal.	Skin	4.1	0.3	0.1	0.0	0.8
58	Lard, 29% cal.	Skin	3.5	0.4	0.4	0.0	1.0
37	Baconfat, 29% cal.	Skin	6.0	0.5	0.1	0.0	1.2
39	Butterfat, 29% cal.	Skin	2.2	0.5	0.1	0.0	1.3
63	Ethyl arachidonate, 1% cal.	Skin	0.7	0.6	0.3	0.7	0.7
53	Methyl linoleate, 1% cal.	Skin	3.7	0.6	0.4	0.0	1.8
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Skin	3.1	0.6	0.3	0.0	1.2
54	Low fat, 1% cal.	Liver	3.6	10.2	0.7	0.0	2.4
59	Low fat, 1% cal.	Liver	4.0	12.6	0.9	0.0	1.5
19	Lard, 29% cal.	Liver	7.3	7.9	6.3	0.5	1.7
35	Lard, 29% cal.	Liver	6.5	8.3	9.1	0.0	3.5
58	Lard, 29% cal.	Liver	5.1	14.0	6.9	0.2	1.9
37	Baconfat, 29% cal.	Liver	7.5	7.4	6.1	0.0	2.8
39	Butterfat, 29% cal.	Liver	3.6	7.2	4.0	0.3	3.2
63	Ethyl arachidonate, 1% cal.	Liver	3.0	14.6	5.0	1.5	2.4
53	Methyl linoleate, 1% cal.	Liver	5.2	11.3	5.2	0.0	2.8
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Liver	6.7	11.4	7.0	0.5	0.8
54	Low fat, 1% cal.	Kidney	3.5	8.2	1.2	0.0	0.3
59	Low fat, 1% cal.	Kidney	4.5	13.5	2.4	0.4	0.1
19	Lard, 29% cal.	Kidney	6.4	8.1	7.7	0.3	0.8
35	Lard, 29% cal.	Kidney	7.0	8.1	8.5	0.8	0.9
58	Lard, 29% cal.	Kidney	5.3	9.2	6.8	0.8	0.0
37	Baconfat, 29% cal.	Kidney	6.0	10.1	8.4	0.2	1.6
39	Butterfat, 29% cal.	Kidney	5.4	7.1	5.6	1.1	1.2
63	Ethyl arachidonate, 1% cal.	Kidney	0.0	13.4	6.3	2.0	0.5
53	Methyl linoleate, 1% cal.	Kidney	6.1	13.2	9.8	0.6	0.2
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Kidney	7.0	10.2	8.3	0.4	0.1

TABLE II—(Continued)

Estimated Unsaturated Fatty Acid Distribution in Per Cent of Total Fatty Acids for Serum, Skin, Liver, Kidney and Heart

Dog	Diet	Tissue	2350Å diene	2700Å triene	3000Å tetraene	3475Å pentaene	3750Å hexaene
54	Low fat, 1% cal.	Heart	6.0	11.9	1.8	0.1	0.8
59	Low fat, 1% cal.	Heart	6.2	16.4	2.3	0.0	1.1
19	Lard, 29% cal.	Heart	7.9	6.2	5.7	0.1	1.0
35*	Lard, 29% cal.	Heart	11.2	9.6	5.5	0.7	1.0
58	Lard, 29% cal.	Heart	15.1	10.9	11.5	0.6	1.1
37*	Baconfat, 29% cal.	Heart	8.3	2.6	2.7	0.3	1.8
39*	Butterfat, 29% cal.	Heart	3.8	3.3	2.3	0.9	1.2
63	Ethyl arachidonate, 1% cal.	Heart	4.3	8.6	2.8	0.0	3.1
53*	Methyl linoleate, 1% cal.	Heart	11.4	7.5	5.6	0.0	2.5
61	Methyl linoleate, 1% cal. + lard, 5% cal.	Heart	15.0	10.4	9.5	0.0	2.8

*Showed decreased iodine number of total fatty acids after storage of tissue.

The method of Holman and Burr (1948) for conjugation of tissue fatty acids was used in order to see if the results for dogs would compare with those for similar tissues in rats. In many instances, the data recorded in Table I compare well with the iodine number and $E^{1\%}_{1cm}$ values reported by Rieckehoff, Holman and Burr (1949) for skin, liver and kidney of rats maintained on a fat-free diet or one containing corn oil. Our estimated percentages of specific fatty acids (Table II) differ considerably from those of Rieckehoff et al. because of different absorption coefficients used as standards in the calculation. In comparable groups of animals, the per cent of tetraene fatty acid calculated for the heart and liver of the dogs are very similar to those reported for rats by Widmer and Holman (1950). Since the fatty acids of blood serum reflect differences in dietary fat to a greater degree than the fatty acids of whole blood, our results for the serum of dogs are strictly not comparable to those of Widmer and Holman for whole blood. Unfortunately they did not give absorption coefficients at 2700Å and 2350Å but interpret their results at 3750Å as indicative of the presence of hexaene fatty acids in rat tissues.

In order to ascertain whether or not the composition of the fats used in the diets was related to the composition of the tissues of animals given lard, baconfat or butterfat, conjugation of these

dietary fats was carried out. Using the 23 per cent KOH-ethylene glycol reagent, the following percentage compositions were found:

	Diene	Triene	Tetraene	Pentaene	Hexaene
Lard (I.N. 65)	5.6	0.7	0.2	0.0	0.1
Baconfat (I.N. 66)	6.2	0.7	0.0	0.0	0.5
Butterfat (I.N. 32)	2.7	0.5	0.3	0.0	0.9

These data show that the lard and baconfat used had very similar compositions. Butterfat, on the other hand, had approximately one-half as much diene fatty acid as lard and baconfat. This difference is of the same order of magnitude as was found for the diene content of serum and other tissues of the animals.

Summary and Conclusions

Spectral analysis of the total fatty acids of blood serum, skin, liver, kidney and heart of dogs maintained on various fat regimens shows the presence of specific unsaturated fatty acids which can be correlated with dietary fat. From the data presented the following trends in the composition of the tissues were observed:

1. The total fatty acids of serum, skin, liver and heart consistently showed the highest per cent of diene fatty acid when fat containing linoleic acid was fed. All the animals receiving these diets showed healthy skin or skin which was improved grossly in appearance over the fat deficient state.

2. The total fatty acids of serum, liver and kidney showed more tetraene fatty acid when diets containing linoleic or arachidonic acid were fed than when the diet was extremely low in fat.

3. The total fatty acids of the heart of all the animals receiving fat or linoleic acid ester showed more tetraene fatty acid than the hearts of animals receiving the low fat diet or ethyl arachidonate.

4. Dogs maintained on a low-fat diet and exhibiting fat deficiency symptoms showed less diene and tetraene fatty acids in serum, skin, liver, kidney and heart than animals with healthy skin.

5. Lard and baconfat contain more diene fatty acid than butterfat. The amount of diene fatty acid in serum, skin and

liver of dogs receiving these dietary fats showed the same relative differences as the fats.

6. The total fatty acids of the serum reflected the composition of the dietary fat to a greater extent than the fatty acids of the skin, liver, kidney or heart.

7. The absorption data at wave lengths of 2700Å, 3475Å and 3750Å have been discussed but their significance cannot be evaluated at the present time.

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FAT IN THE DIET IN RELATION TO NUTRITION OF THE DOG

IV. Histologic Features of Skin From Animals Fed Diets With and Without Fat*

Arild E. Hansen, S. Grant Holmes,† and Hilda F. Wiese

The purpose of this report is to present data concerning histologic features of the skin of dogs having fat in the diet and of the skin of dogs fed diets lacking fat. These observations were made in order to integrate the microscopic and gross appearance with the results of the chemical and spectral analyses of the skin of the animals.

Materials and Methods

Histologic examination has been made on a total of sixty skin specimens from twenty-eight dogs. The specimens were taken from comparable areas on the dorsal surface of the thigh either by excision under local anæsthesia or at the time of autopsy. The sections including tissue to the subcutaneous fatty layer were placed on wooden strips to prevent curling and immediately immersed in freshly prepared Susa's fixative* for about 12 hours. They were then run through the usual alcohol series of 70, 95 and 100 per cent and cleared in methyl benzoate. Next they were infiltrated with paraffin for about four hours, blocked and sections cut six microns in thickness. Staining was with hemotoxylin and mercurochrome.

Results

To facilitate presentation of the typical histologic findings, the skin of the control animals receiving lard in the diet and

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†Present address: Pensacola, Florida.

*Susa's fixative consists of mercuric chloride 4.5 g., sodium chloride 0.5 g., formalin 20 ml., water, 80 ml., glacial acetic acid 4.0 ml., trichloracetic acid 2.0 g.

exhibiting healthy skin will be described first. A similar description will be made of the skin of dogs showing characteristic fat-deficiency symptoms (Hansen and Wiese, 1951).

Control Dogs Receiving Lard (30 Per Cent of Calories in Form of Fat)

Low power magnification 100X: The *stratum corneum* has a thin, clear, lace-like structure. The *epidermis* is 2 to 3 cell layers in thickness and is uniform throughout. The *dermis* consists of sharply demarcated and loosely woven collagen bundles. The dermal fat cells are well defined. The hair follicles have epidermal cells 2–3 layers in thickness with a slightly expanded bud. The hair shafts are intact. The sebaceous glands consist of alveoli with compact cells.

High power magnification 440X: The *stratum corneum* reveals no nuclei present in the desquamated cells. In the *epidermis* the nuclei of the cells tend to be oriented parallel to the surface. In the *dermis*, the endothelial cells are thin. Slight round cell infiltration is evident.

Oil immersion 970X: The cell borders are approximated, the cytoplasm appearing syncytial. No prickling is evident.

Dogs Reared on Low Fat Diet—Fat One Per Cent of Calories

Low power magnification 100X: The *stratum corneum* is definitely heavier than the cellophane-like appearance of this layer in the dogs receiving 30 per cent caloric fat. The *epidermis* is increased to 6 to 12 cell layers. The inner margin is uneven and shows extensive pegging. The *dermis* is thickened, the collagen bundles appear swollen with evidence of edema. Round cell infiltration is very marked. The epidermal cells of the hair follicles are increased to 8 to 12 cell layers, the buds are expanded. Many hair shafts are plugged. The dermal fat is indistinct.

High magnification 440X: In the *stratum corneum* there is hyperkeratosis with many nuclei present in the cells. The parakeratosis shows marked disruption of the stratum disjunctum by scaling associated with the development of the stratum

granulosum and lucidum. In the *epidermis* the nuclei of the cells in the first 2 to 3 layers lie parallel to the surface; however in the basilar portion the nuclei assume a vertical formaiton with palisading of cytoplasm and evidence of marked proliferation, driving epidermal pegs into the dermis. The *dermis* appears edematous, the endothelial cells are swollen and the lumina are greatly reduced in size. Hyperkeratinization of the hair follicles is evident and sometimes there is papillary formation. In many instances the hair shafts appear atrophic. Early, the sebaceous glands show large alveoli with active cells. Much later, the glands appear shrunken.

Oil immersion 970X: In the *epidermis,* there is loss of syncytial appearance of the cytoplasm and evidence of prickling is abundant.

Photomicrographs of the skin of young dogs reared on the control diet containing 30 per cent caloric fat and on the low fat diet are presented in Figures 1 to 4.

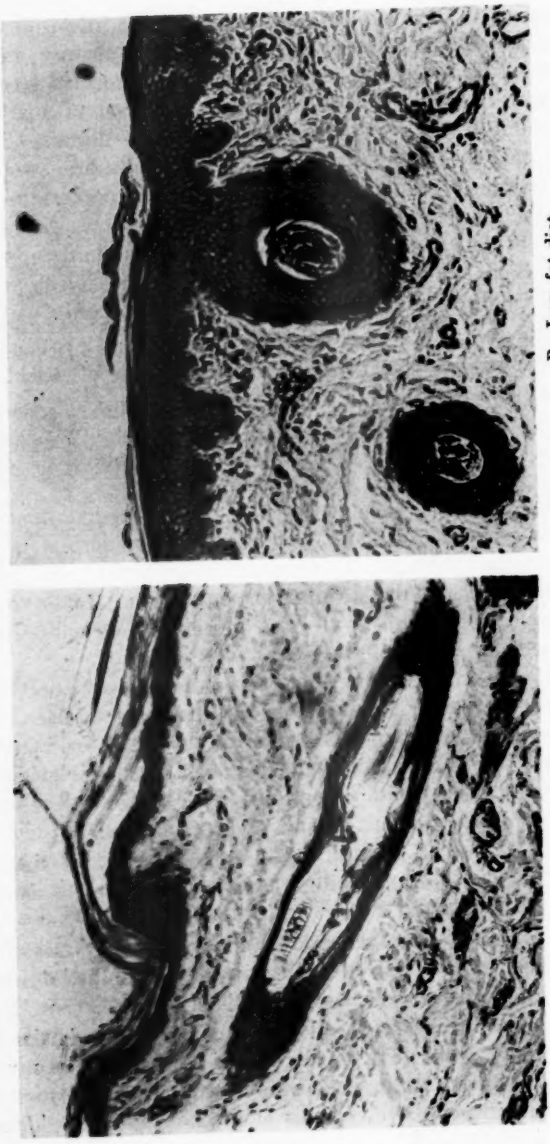
In Figure 1A can be noted the thin cellophane-like appearance of the stratum corneum, the even, thin epidermal layer and normal hair follicles. In contrast, in Figure 1B one may note the denseness of the stratum corneum with parakeratosis, the thickened epidermis with peg formation, atrophic hair follicles and round cell infiltration.

Figure 2, under oil immersion, represents the same sections as in Figure 1. In Figure 2A, again, one sees the lace-like appearance of the stratum corneum and the well defined structure of the epidermis without evidence of protoplasmic bridges. In Figure 2B, the stratum corneum is compact and the entire structure is poorly defined. Prickling is evident.

Figures 3A and 3B show differences in the relative size of the sebaceous glands in the skin of a control dog and one reared on a low fat diet.

Figure 4A illustrates a normal capillary in the dermis of a control dog. Figure 4B illustrates swollen endothelial cells resulting in a small lumen in the dermis of a fat deficient dog.

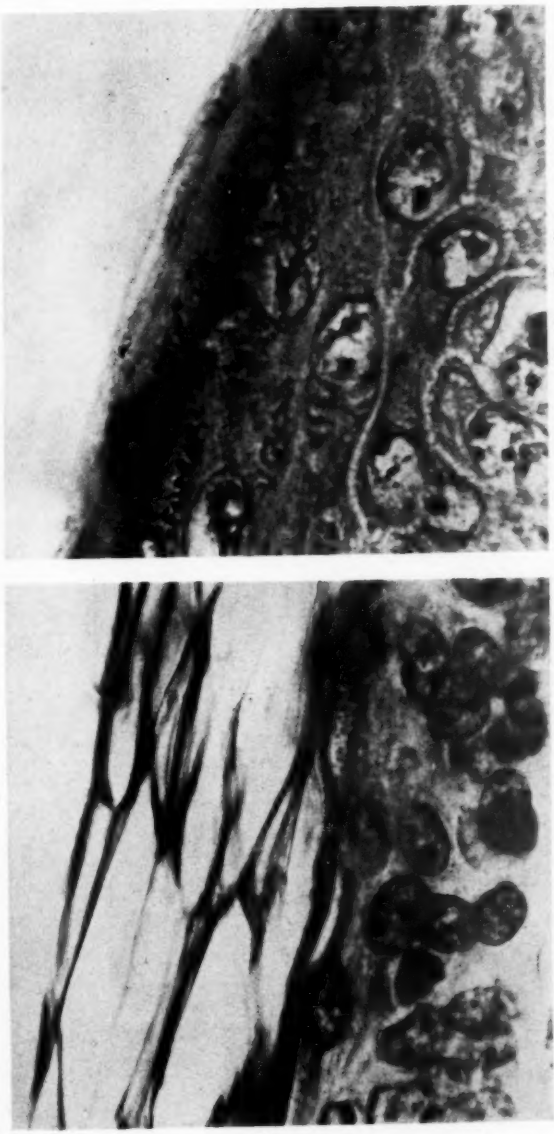
Variations in the rate of development of the histologic alterations have been noted. Early, there is an increase in the number of cell layers in the epidermis with palisade formation and a thickening of the epidermis of the hair follicles. These



B. Low fat diet.

A. Control diet.

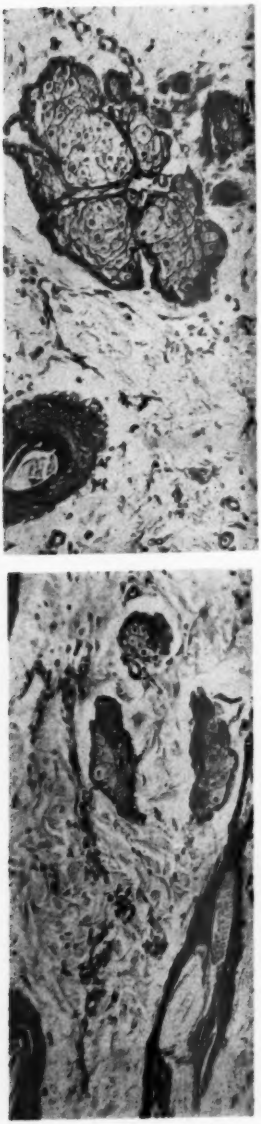
FIG. 1. Magnification 100X—Epidermis of Dogs.



B. Low fat diet.

A. Control diet.

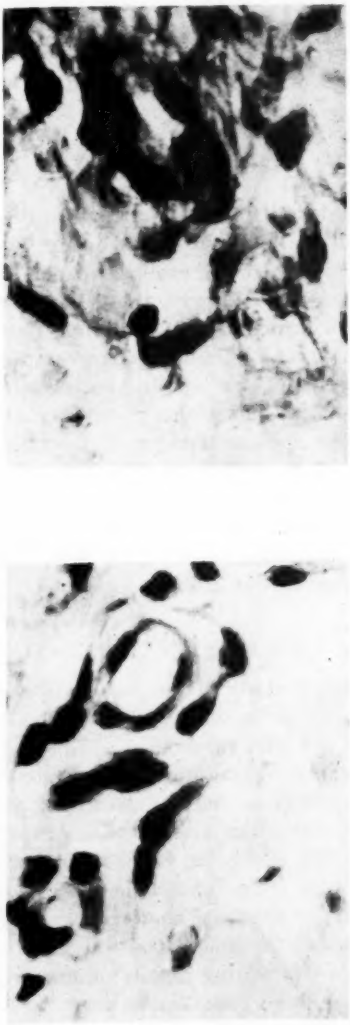
FIG. 2. Magnification 1425X—Epidermis of Dogs.



B. Low fat diet.

A. Control dog.

FIG. 3. Magnification 100X—Sebaceous glands.



B. Low fat dog.

A. Control dog.

FIG. 4. Magnification 855X—Endothelial cells.

changes may occur before desquamation or loss of hair are noted grossly. Later parakeratosis, pegging, plugging of the hair follicles and enlargement of the sebaceous glands become evident. Brief descriptions of the gross and microscopic appearance of the skin of six puppies are presented in Table I.

Changes Observed Following Feeding of Fat to Deficient Dogs

After fat was added to the diet of a dog showing evidence of deficiency, structural alterations in the horny layer, epidermis and dermis were observed. A number of changes appear to take place simultaneously. Early, there is a decrease in the thickness of the epidermis and the epidermal layer of the hair follicles; peg formation is lessened, parakeratosis disappears and the stratum corneum becomes thin and lace-like in appearance. Cellular material and edema persist for some time. As the thickness of the epidermal layer of the hair follicles decreases, plugging of the follicles is lessened and regeneration of hair can be observed. About the same time, the sebaceous glands become more nearly normal in size. The types of change which take place and the rate at which these are seen microscopically are shown for six animals in Table II.

Discussion

It is evident from a study of the histologic structure of the skin of dogs maintained on diets with or without fat that the absence of fat in the diet materially affects the microscopic appearance of the skin. Alterations are demonstrable in the horny layer, the epidermis and the dermis of animals reared on a fat deficient diet. Rice and Jackson (1934) studied the histologic distribution of fat in various tissues including the skin of rats suffering from fat deficiency and found no apparent change in the amount, character and distribution of the visible (stainable) fat in the trachea, lung and skin. No mention was made concerning details of the histologic structure of the skin of these rats. Some years later Williamson (1941) reported changes in the epidermis of rats reared on fat-free diets. He described the epidermis of the rat on a

TABLE I

Comparison of Gross and Microscopic Appearance of Skin of Dogs on Low Fat Diet

Dog	Duration of diet (months)	Gross	Microscopic		
			Stratum Corneum	Epidermis	Dermis
66	3	Moderate desquamation on ventral surface, no desquamation on back. Hair dry.	Some thickened areas.	Slight thickening in some areas, 2–5 cells, poor differentiation.	Edema, cellular infiltration, some thickening of epidermis of hair follicles, small sebaceous glands.
67	3	Desquamation on dorsal and ventral surfaces. Dry hair.	Thick, no parakeratosis	2–5 cell layers. Thickening with palisade formation, very poor differentiation.	Edema, cellular infiltration, more thickening of hair follicles than #66. Sebaceous glands normal size.
68	3	Moderate desquamation on ventral surface.	Same as #66.	Same as #66.	Same as #66.
70	3	Good, no desquamation evident.	Normal.	Slight thickening in few areas.	Normal.
59	3	Loss of hair on head, legs and ventral surface, red skin, numerous infected areas, extensive peeling on dorsal surface. Hair coarse, dry and matted. Feet red, swollen.	Thick.	Thickened, 5–8 cell layers, palisading and pegging.	Broken collagen, extensive cellular infiltration, large sebaceous glands, plugged hair follicles.
59	5½	More peeling, redness and loss of hair.	Some parakeratosis.	5–8 cell layers, extensive peg formation.	Collagen edematous, great deal cellular material, swollen endothelial cells, hair in some shafts but others appear atrophic.

Note—Dogs #66, 67, 68, 70 litter mates. Dogs #59, 60 litter mates.

TABLE I—(Continued)

Comparison of Gross and Microscopic Appearance of Skin of Dogs on Low Fat Diet

Dog	Duration of diet (months)	Gross	Microscopic		
			Stratum Corneum	Epidermis	Dermis
59	10	Alopecia of ventral surface, ears, head, legs. Coarse, matted hair on back. Infected ears, reddened paws, general emaciation.	Extensive parakeratosis.	5–8 cell layers, marked pegging, cells more compact than at 3 months.	Edema, cellular material, shrunken sebaceous glands, degenerative appearance of hair follicles.
60	3	Loss of hair on head, legs and ventral surface. Extensive peeling. Hair on back dry and coarse. Feet red, edematous. Ears infected.	Marked parakeratosis.	5–10 cell layers, palisade and peg formation. Poor differentiation of cells.	Broken collagen, extensive cellular infiltration, plugged hair follicles, destruction in follicles, swollen endothelial cells, large sebaceous glands.
60	5	Severely deficient with emaciation, alopecia and infected ears and body.	Parakeratosis.	Same as 3 months, but cells more compact.	Very extensive edema, hair follicles more atrophic than at 3 months, sebaceous glands normal in size.
60	6	Severely deficient.	Same as 5 months.	Same as 5 months.	Generalized degeneration, shrunken sebaceous glands.

Note—Dogs #66, 67, 70 litter mates. Dogs #59, 60 litter mates.

TABLE II

Histologic Features of Skin of Dogs Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Stratum Corneum	Epidermis	Dermis
43	Low fat	36	Parakeratosis	8–12 cell layers, palisading, peg formation, prickling.	Marked edema, cellular infiltration, swollen endothelial cells, hyperkeratinization of hair shafts. Sebaceous glands shrunken.
43	Lard, 10% cal.	12	Normal	2–4 cell layers, occasional peg.	Edema, nearly normal keratinization of hair follicles, some regeneration of hair.
55	Low fat	17	Thick	8–10 cell layers palisading, pegging, prickling.	Edema, round cell infiltration, large sebaceous glands, swollen endothelial cells.
55	Lard, 15% cal.	5	Normal	3–5 cell layers, no pegging.	Edema, but decreased round cell infiltration. Regeneration of hair.
58	Low fat	3½	Parakeratosis	6–10 cell layers, palisading, extensive pegging.	Round cell infiltration, fragmented collagen, large sebaceous glands, proliferation of hair follicles.
58	Lard, 29% cal.	1	Lessened parakeratosis, some lace-like keratin.	3–6 cell layers, lessened pegging.	Less cellular infiltration, collagen less fragmented. Regeneration of hair.
58	Lard, 29% cal.	3	Normal	3–4 cell layers, sharp differentiation, essentially normal keratinization.	Some cellular material. Collagen essentially normal. Hair follicles, 3–4 epidermal cells, regeneration of hair. Sebaceous glands nearly normal.

TABLE II—(Continued)

Histologic Features of Skin of Dogs Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Stratum Corneum	Epidermis	Dermis
58	Lard, 29% cal.	4	Normal	2–3 cell layers, occasional peg.	Collagen normal, good growth of hair, sebaceous glands normal.
58	Lard, 29% cal.	6	Normal	2–3 cell layers, occasional peg, good differentiation of cells.	Good regeneration of hair. Normal collagen, many cell fragments.
53	Low fat	17	Marked parakeratosis	6–10 cell layers, palisade formation, marked pegging.	Collagen edematous, extensive cellular infiltration, swollen endotheial cells, hyperkeratosis of hair follicles, 6–10 epidermal cell layers, large sebaceous cells.
53	Methyl linoleate, 1% cal.	¼	Same as low fat	Same as low fat.	Same as low fat.
53	Methyl linoleate, 1% cal.	2	Almost normal keratinization	2–5 cell layers, some palisading and peg formation.	Few enlarged sebaceous cells, regeneration of hair, 2–3 cell layers on follicles.
53	Methyl linoleate, 1% cal.	3	Normal	2–4 cell layers, remnants of palisading.	Sebaceous glands about normal, collagen fragmented, round cell infiltration, good growth of hair.
53	Methyl linoleate, 1% cal.	4½	Normal	2–4 cell layers, occasional peg.	Broken collagen, normal sebaceous glands, good growth of hair.
61	Low fat	3½	Marked parakeratosis	8–12 cell layers, extensive palisade and peg formation.	Collagen destruction, cellular infiltration, swollen endothelial cells, large sebaceous glands, hyperkeratosis of hair follicles with plugging.

TABLE II—(Continued)

Histologic Features of Skin of Dogs Before and After the Addition of Fat to the Diet

Dog	Diet	Duration of diet (months)	Stratum Corneum	Epidermis	Dermis
61	Methyl linoleate, 1% cal.	1	Thick but little parakeratosis	3–7 cell layers, palisading, minimal pegging.	Edema and cellular infiltration, hair follicles 2–3 epidermal cells, regeneration of hair.
61	Methyl linoleate, 1% cal.	3½	Normal	2–4 cell layers, no peg formation, sharp differentiation of cells.	Normal sebaceous gland. Extensive degeneration of hair but evidence of new hair, considerable cellular material.
61	Methyl linoleate, 1% cal.	4¼	Normal	Thickening in some areas, 2–5 cell layers, loss of differentiation.	Degeneration of hair follicles, thickening of epidermal layer, numerous sebaceous glands.
61	Methyl linoleate, 1% cal. + lard, 5% cal.	6 1½	Normal	2–5 cell layers, thickened in some areas.	Considerable cellular material, normal endothelial cells, atrophic material in hair shafts.
63	Low fat	3½	Parakeratosis	8–12 cell layers, palisade and peg formation.	Broken collagen, large sebaceous glands, cellular infiltration, swollen endothelial cells, epidermal layer of hair follicles thickened, degeneration of hair.
63	Ethyl arachidonate, 1% cal.	1	Thin, nearly normal	4–8 cell layers palisading, some peg formation.	Collagen edematous, extensive cellular material. Many hair shafts plugged but definite regeneration of hair in some shafts, large sebaceous glands.
63	Ethyl arachidonate, 1% cal.	1½	Same as above	Same as above.	Same as above.

normal diet as being very thin, 1 to 2 cells in thickness. The epidermis of the rat on a fat-free diet was somewhat thicker (2–3 layers of cells) and showed more differentiation than that of the normal rat. The stratum granulosum was well developed and the stratum corneum was thicker in places than the rest of the epidermis. There was slightly greater infiltration of cells in the skin of fat-free animals than in the control animals. When linseed oil was added to the diet of the fat-free rat, the histologic structure of the skin was similar to that of the normal rat. In dogs, histologic alterations in the skin resulting from the lack of dietary fat are much more extensive than those reported to be present in rats on fat deficient diets. This confirms our impression that abnormalities of the skin resulting from the dietary lack of fat are more pronounced in dogs than in rats. It is apparent that the skin abnormalities are not related to any derangement in the metabolism of vitamin A inasmuch as the dietary intake and the blood levels of vitamin A for the deficient dogs are the same as for the control group.

The first histologic change indicating a lack of dietary fat in dogs is an increase in the number of cells in the epidermis and epidermal layers of the hair follicles. This is followed by an increase in the horny layer and an increase in the activity of the sebaceous glands. Analysis of the desquamated material obtained from the skin of fat deficient dogs shows it to have very high percentages of cholesterol and cholesterol ester. The total fatty acid fraction of this portion of skin has an extremely low iodine number. The question arises, is the increased activity of the sebaceous glands and the elaboration of cholesterol and saturated fatty acid a physiological adjustment made by the animal to meet its essential need for fat? The greasy skin of some fat deficient dogs and the oily hair of animals recovering from the deficiency state can be explained readily by increased activity of the sebaceous glands. Plugging of the hair follicles could easily explain the alopecia observed. Whether specific fatty acids are needed for structural purposes, normal maturation of certain cells, or are required metabolically, for example, in enzyme systems, is not apparent at present. However, spectral analysis of the fatty acids of the skin of dogs with and without dietary fat show

that the skin of animals not receiving fat has a smaller amount of the two double bond fatty acids than control animals having fat in the diet (Wiese and Hansen, 1951). Certainly the accumulation or proliferation of the cells of the epidermis, in the absence of dietary fat, indicates a specific need for fat not synthesized by the dog.

In the dermis of the fat-deficient animals, the swelling of the endothelial cells is extensive. If this is a significant feature in regard to pathogenesis, the consequent decreased blood supply may be related to the edema and possibly to the swelling of the collagen fibers. The meaning of the excessive amount of cellular material in the dermis is not clear. It may represent ordinary round cell infiltration, as seen in a variety of conditions; on the other hand, it may be a matter of cells sloughing into the tissue because of the tremendous crowding of the epidermal cells.

Remarkable as the histologic derangement may be in the skin tissue, it seems extraordinary that even with the lapse of years (up to six years in our experience) the microscopic alterations are rectified when adequate fat is included in the diet. We have no reason whatsoever to suggest that any of the histologic changes are irreversible. In order to understand the nutritional significance of dietary fat in relation to the condition of the skin it seems apparent that greater detailed histologic study of the cellular changes is warranted.

Experimental results showing good correlation between the unsaturated fatty acid content of the serum and the gross and microscopic appearance of the skin of dogs fed diets with and without fat should be helpful in the interpretation of findings in a study of the human requirement for fat.

Summary and Conclusions

Definite alterations from those of control animals occur in the histologic structure of the skin of dogs which have been deprived of dietary fat. Changes develop in the stratum corneum, epidermis and dermis.

In control dogs receiving dietary fat containing linoleic and/or arachidonic acid and exhibiting a healthy skin, the

stratum corneum is thin and lace-like in appearance. The epidermis has a thickness of 2–3 cell layers and shows good differentiation. The dermis has well defined collagen bundles, normal hair follicles, thin endothelial cells with large lumina and slight cellular infiltration.

In the fat deficient dog showing distinct abnormalities of the skin including desquamation and loss of hair, the stratum corneum is compact and may show parakeratosis. There is thickening of the epidermis with palisade and peg formation. In the dermis, there is evidence of extensive edema and the presence of cellular material, fragmented collagen, swollen endothelial cells with small lumina, hyperkeratinization of the hair follicles, plugging of the hair shafts, degeneration of the hair and enlarged sebaceous glands. After prolonged fat deficiency, the sebaceous glands appear shrunken.

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EFFECT OF THYMIC EXTRACT ON A TRANSPLANTED SARCOMA IN RATS

JOHN B. LOEFER AND NORBERT G. GILLES*

There are a number of accounts in the literature which indicate that certain tissue extracts affect the growth of tumors. Materials obtained from the spleen, for example, were reported to have had an inhibitory effect on tumors (Lewisohn, 1938; Lewisohn et al., 1940) and on tumor cells (Diller and Watson, 1949). Testis extracts have also been shown to inhibit development of lymphosarcoma in mice (Arnesen, Buxton and Dulaney, 1949).

The effect of thymic extract on tumors seems not to have been investigated, although there is considerable evidence which indicates that thymectomy reduces the incidence of spontaneous leukemia. McEndy, Boon and Furth (1944) noted that incidence in Ak mice fell to approximately 10% in thymectomized animals, whereas near 70% of control animals developed the disease. These findings were later verified by Furth (1946). Law and Miller (1950) observed a striking reduction in incidence of spontaneous lymphoid leukemia following early thymectomy in mouse strains RIL, C58 and dba. Radiation-induced tumors were also fewer in thymectomized C57 black mice (1 in 23 = 4%) than in normal animals (10 in 21 = 48%), according to Kaplan (1950). In view of these reports, one might expect that injection of thymic extract would affect the leukemic or tumorogenic process, and hence the following experiments were carried out to test this hypothesis.

Materials and Method

A thymic extract, kindly furnished by Dr. Maynard Murray,[1] was used. The material was essentially an isotonic saline extract prepared without heating from lyophilized glands of

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[1]The Murray Foundation, Cincinnati, Ohio.

fetal calves. It was sterile, pyrogen-free, pH was 7.4 and each milliliter contained the equivalent of 0.05 gram of fresh thymus gland.

Test groups of rats were injected daily either intravenously or intraperitoneally with the extract for several days before implantation with subcutaneous grafts of the fibrosarcoma used in earlier experiments (Loefer and Gilles, 1951). Rats from our own colony, descendants of Sherman strain stock, maintained on a diet of Purina Laboratory Chow, were used. The percent of tumors that developed was taken as the criterion of effect.

Results

Thirty-three seven-week animals, averaging 130 grams in weight, were used in experiment I. Ten males and eight females were daily injected intravenously with 0.3 milliliter of the thymic extract for three days; two days later injections were resumed and continued for four days. At this time all test animals, as well as seven male and eight female controls which had received no thymic extract, were implanted with grafts from the peripheral material of an 18-day stock tumor. Seven more thymic extract injections were given to the test animals during the next eleven days, bringing the total amount of injected material to 4.2 milliliters. When the tumors were 18 days old, the percentage which had developed in both groups was noted and recorded as shown in Table I.

TABLE I

Effect of Thymic Extract on Development of Rat Tumor Grafts

	No. rats grafted	No. of takes	Percentage takes
Controls:			
Exp. I.	18	4	22.2
Exp. II.	17	3	17.6
Total or average	35	7	20
Thymus-treated:			
Exp. I.	15	8	53.3
Exp. II.	26	11	42.3
Total or average	41	19	46

A second experiment was performed with 43 five-week rats, which averaged 106 grams in weight. Eight males and nine females comprised the control group; 13 males and 13 females the test group. Each of the latter received one milliliter of thymic extract intraperitoneally daily for four days, after which portions of the same 16-day stock tumor were grafted subcutaneously with a trochar into all animals. After a four-day interval, four more consecutive daily injections of the extract were given to the test animals, so that each rat received a total of 8 milliliters of the material. Control rats were given one-milliliter intraperitoneal injections of physiological saline on corresponding days. Fourteen days after tumor implantation the tumors were palpated and the results recorded as in Table I.

Totals for both experiments reveal that there were 20% takes in the controls as compared with 46% in the thymus-treated animals. Chi square calculations on these results indicate that the value of "p" is less than 0.02. Weight increase for control animals in the two series was 33%, whereas treated animals increased 39%. Incidence in males and females was similar. The tumors were not measured, but there was no apparent difference in the mean size of those in each group.

Discussion

While it is clear that incidence of tumor takes was higher in the treated animals, it is not known whether this increase reflects a response to nutritive or hormonal factors. Indeed, Schaffer, Ziegler and Rowntree (1938) presented evidence to indicate that the striking effects of Hanson's (1929) thymic extract on growth and early maturation of rats earlier reported by Rowntree et al. (1934, 1935), and Rowntree (1935), may have been due to the action of such biological substances as cysteine, glutathione and ascorbic acid. It should be noted, however, that Andreasen and Hamburger (1948) reported that the experiments of Rowntree et al. were not verified in other laboratories either with supplementary gland extracts or thymic implants.

No analysis of the nutrient material of Murray's extract was available, but certain observations by Dr. Fred W. Oberst

(personal communication) on mice treated with the same extract indicate that it is rich in nutrient elements. It probably contains an abundance of nucleoprotein derivatives. It is quite possible that the extract supplemented marginal quantities of certain essential materials, and in this manner was responsible for increasing the percentage of takes. In line with this finding is evidence that supplementary pyridoxine may raise the level of successful tumor implants (Loefer, 1951).

Summary

The percentage of takes of transplanted rat fibrosarcoma grafts was significantly greater in rats which had received injections of a bovine fetal thymic extract than in controls. The substance used (Murray) was a pyrogen-free saline extract prepared without heating from lyophilized fetal glands and contained the equivalent of 0.05 gram of thymus per milliliter. It was administered either intravenously or intraperitoneally to animals five and seven weeks old for several days previous to implantation of tumor stock. Controls received injections of physiological saline on the same days. Grafts in seven of 35 controls (20%) developed, whereas in treated animals 19 of 41 (46%) had well-developed tumors after 14–18 days. Beneficial results may have been due to supplementation of marginal nutritive or growth-promoting factors.

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THE EFFECT OF ADRENAL CORTICAL STEROIDS ON CIRCULATING ANTIBODIES IN X-RADIATED ANIMALS*

ROLAND B. MITCHELL,† JOHN D. FULTON,‡ JOHN M. TALBOT,§ AND ALLYN W. KIMBALL‖

The detrimental effects of x-radiation on antibody production are well known. Hektoen demonstrated that the production of circulating antibodies was reduced markedly when animals were exposed to the effects of x-radiation at the time of injection of antigen. This reduction in antibody production was attributed to a destructive action by the x-radiation of lymphatic tissues and marrow. Murphy and Sturm employed x-radiation to deplete animals of lymphatic tissue and reported that the production of protective antibodies was decreased as compared with control animals. Other recent work has emphasized that lymphoid tissue is implicated in the circulating antibody response of an animal to antigen. Kass and Ehrich and Harris and White and Dougherty have concluded that lymphocytes contain gamma globulin and may be potential carriers of antibody protein. Ehrich later reported that antibody production is accomplished by the plasma cell in the lymph node.

Dougherty, White, and Chase have suggested that adrenal cortical hormones exercise a control over the relative number of circulating lymphocytes. These workers reported that a nonspecific stimulation of the adrenal cortex resulted in degenerative changes in lymphoid tissue and, also, in diminished lymphocyte production. They concluded that this breakdown of lymphocytes resulted in a release of gamma globulin and in immunized animals produced an increased concentration of circulating antibody. These investigators found that injection of C-11 oxygenated cortical steroids at the time of antigen injection resulted in a higher antibody level than in control

*From the Departments of Aerobiology, Radiobiology, and Biometrics, USAF School of Aviation Medicine, Randolph Field, Texas. Received for publication April 15, 1951.

†Head, Department of Aerobiology.
‡Major, USAF (MSC), Department of Aerobiology.
§Colonel, USAF (MC), Head, Department of Radiobiology.
‖Department of Biometrics. Present address: Oak Ridge National Laboratory.

animals. They found that this antibody titer could be maintained at a higher level when repeated injections of the hormone were administered.

Certain subsequent workers have been unable to confirm these studies. Eisen, et al. showed that, under the conditions of their studies, the administration of adrenal cortical substances did not influence the level of circulating antibodies or the serum globulin concentration. They concluded that adrenal cortical activity is not essential for either the fabrication or release of antibodies and gamma globulin. De Vries reported that the administration of adrenocorticotrophic hormone to previously immunized animals failed to produce a rise in circulating antibody titers although a decrease occurred in the number of circulating lymphocytes. Fischel and co-workers were unable to demonstrate that administration of adrenocorticotrophic hormone or of x-ray had any effect on circulating antibody titers in previously immunized animals.

Craddock and Lawrence investigated the effect of roentgen irradiation on antibody formation in rabbits and concluded that the depressant effect of x-ray on antibody production could not be related entirely to a depression of lymphoid tissue. These workers found a marked difference in the effect of x-ray on antibody formation depending upon whether the irradiation was given before or after the beginning of antibody production. They suggested that reserve sources of tissue are capable of accomplishing antibody formation once it has begun even though a major portion of the lymphoid tissue has been damaged.

The present studies were undertaken in an attempt to point the way to further research in enhancing antibody production from a reticulo-endothelial system damaged by exposure to x-radiation. It was considered worth while to investigate the effect of adrenal cortical extracts when administered to x-radiated animals possessing high circulating antibody titers to a selected antigen. In such animals any definite change in the antibody content should be more easily detected than in animals with low concentrations of circulating antibodies.

The first portion of this study was planned as an exploratory experiment designed to investigate the effect of x-radiation, additional injections of antigen, and the administration of adrenal cortical extract on the circulating antibody content of

rabbits with high agglutinating titers to *Salmonella typhosa* antigen. A factorial design was chosen for this preliminary experiment because, in general it provides factor comparisons of greater accuracy and with fewer animals than other designs which might be considered applicable.

The second experiment was planned to focus primarily on the effects of adrenal cortical extracts when administered to animals possessing high circulating agglutinating antibody titers to a selected bacterial antigen at the time of x-radiation. The factor of additional injections of antigen to the immunized animals was omitted in the second experiment.

Materials and Methods

Experiment Number 1

Sixteen female rabbits immunized to a state of high circulating antibody titers with heat-killed *Salmonella typhosa* antigen were used in this experiment. A control period of 2 to 3 days was established for all rabbits prior to establishing the experimental conditions, during which time circulating antibody titers were determined for each of the experimental animals.

From Chart 1 it may be seen that in this experiment there are eight possible treatment combinations, one set of which is called a replication. This experiment employed two replications.

The immunized rabbits in each of the two replications were treated in the following manner. Animals which received additional antigen were injected intravenously with 1.0 ml. of antigen containing two billion killed *Salmonella typhosa* organisms. The rabbits to which adrenal cortical extract[1] was given received 5.0 ml. intraperitoneally at the 0-, 6-, 12-, and 30-hour periods following the start of the experiment.

Whole body, 200 kv. x-radiation was given at the 0-hour period (start of the experiment) in the amount of 500 r, at the rate of 20 r per minute, with filtration through 1.0 mm. aluminum + 0.50 mm. copper and 0.25 mm. copper inherent in the tube.

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[1] "Eschatin," a suprarenal cortex extract, was employed (Parke, Davis and Co.). Each 1.0 ml. contains 50 dog units.

Sufficient whole blood specimens (approximately 3 to 4 ml.) to provide serums for agglutination tests were obtained from ear veins at the beginning of the experiment (0 hours) and then at intervals of 2, 4, 6, 8, 10, 12, 14, 18, 24, 30, 48, and 498 hours.

CHART 1. TREATMENT COMBINATIONS EMPLOYED

EXPERIMENT 1				
GROUP DESIGNATION	A	B	C	NUMBER of IMMUNIZED RABBITS
	ANTIGEN	ADRENAL CORTICAL EXTRACT	X-RADIATION	
(1)	0 *	0	0	2
a	+ **	0	0	2
b	0	+	0	2
c	0	0	+	2
ab	+	+	0	2
ac	+	0	+	2
bc	0	+	+	2
abc	+	+	+	2

EXPERIMENT 2				
GROUP DESIGNATION	A	B		NUMBER of IMMUNIZED RABBITS
	X-RADIATION	ADRENAL CORTICAL EXTRACT		
(1)	0	0		9
a	+	0		9
b	0	+		10
ab	+	+		10

* 0 = Absence of factor
** + = Presence of factor

The agglutination tests were done at random and in triplicate for each serum studied. A macroscopic tube test was used to determine the circulating antibody titers. Dilution increments of 1:500 (e.g., 1:9,000, 1:9,500, 1:10,000) were employed.

Experiment Number 2

Thirty-eight female rabbits immunized to high circulating antibody titers with *Salmonella typhosa* antigen were distributed within the groups of the experimental design as shown in chart 1.

The rabbits to which adrenal cortical extract[2] was given (nine exposed to x-radiation and ten with no x-ray exposure) each received 5.0 ml. into the skin of the back one hour before any of the animals received x-radiation and then received 1.0 ml. subcutaneously each day for the next 14 days. Whole body x-radiation exposure was given under the conditions described for experiment number 1.

Whole blood specimens (approximately 5.0 ml.) to provide serum for agglutination tests were obtained from ear veins of all animals on the day prior to x-radiation and again at 1, 5, 8, 14, 20, 27 and 34 days following x-radiation.

Macroscopic tube agglutination tests were used to determine the circulating antibody titers. Dilution increments of 1:500—beginning with a dilution of 1:100, then 1:500, and continuing through a dilution of 1:12,500—were employed.

Results

Experiment Number 1

The effects on ciculating antibody titers of the absence or presence of each of three factors, namely, x-radiation, adrenal cortical extract, and antigen were observed in sixteen immunized rabbits.

The data presented are the differences between measurements at the beginning of the observation period and those obtained at the various sampling intervals. In the following statement of results the plus (+) values indicate increases and the minus (—) values decreases from the beginning levels obtained for the circulating antibody titers. The results presented in tabular form are certain mean values of the measurements which show the responses of the animals during the 21-day period.

[2]Lipo-Adrenal Cortex in cottonseed oil; the Upjohn Company. Each ml. contains 40 rat units (survival-growth) and is equivalent in biological activity to 1.0 mg. of 17-hydroxycorticosterone; chlorobutanol, 5 mg.

The average changes in antibody titers over the entire period of observation (0–498 hours), grouped according to treatment, are shown in Table 1.

TABLE 1. EXPERIMENT 1.

AVERAGE CHANGES IN CIRCULATING ANTIBODY TITERS IN THE PRESENCE OR ABSENCE OF ANTIGEN, ADRENAL CORTICAL EXTRACT, and X-RADIATION

Difference between zero hours and:	GROUPS					
	ANTIGEN		ADRENAL CORTICAL EXTRACT		X-RADIATION	
	Administered	Withheld	Administered	Withheld	Administered	Withheld
2 hours	+ .10	− .92	− .65	− .16	− 1.06	+ .25
4 "	+ .42	− .96	− .56	+ .02	− .60	+ .06
6 "	− .17	− 1.08	− 1.06	− .18	− 1.65	+ .40
8 "	+ .23	− .60	− .27	− .10	− .65	+ .27
10 "	+ 1.42	− .54	+ .91	− .04	− .25	+ 1.12
12 "	+ .60	− .31	+ .40	− .10	− .60	+ .90
14 "	+ .67	− .71	+ .42	− .46	− 1.15	+ 1.10
18 "	+ .18	− 1.48	+ .12	− 1.42	− 1.38	+ .08
24 "	− 1.08	− 1.73	− 1.29	− 1.52	− 2.60	− .21
30 "	− 1.02	− 1.44	− 1.17	− 1.29	− 2.54	+ .08
48 "	− 2.71	− 1.25	− 2.13	− 1.83	− 4.17	+ .21
498 "	− 2.38	− 1.25	− 1.92	− 1.75	− 4.29	+ .62

Note: The titers were measured in thousands (e. g. 1.0 represents 1:1,000). The values tabulated are means of differences between measurements of circulating antibody titers at the beginning of this experiment and at indicated times during the period of study. The plus (+) values indicate increases and the minus (−) values indicate decreases from the beginning levels obtained for the circulating antibody titers.

It appears that the animals which received additional antigen showed a slight increase in antibody titer and a subsequent decrease toward the end of the 498-hour period. Animals which did not receive additional antigen showed no regular changes. However, none of the differences at any of these intervals is statistically significant. Apparently the rabbits possessed a relatively stable high immunity prior to the beginning of the experiment. No differences between the animals which received adrenal cortical extract are revealed in Table 1, although it is quite clear that the animals which received x-radiation showed a substantial decrease in titer toward the end of the observation period, whereas the animals not exposed to x-radiation showed no such decrease. The differences in antibody titers between the x-radiation group and the group without x-radiation at the 48- and 498-hour periods are significant at the 5 percent level.

More important conclusions may be drawn from an examination of Table 2. Here the results for the x-radiation group

TABLE 2. EXPERIMENT 1

AVERAGE CHANGES IN CIRCULATING ANTIBODY TITERS IN THE PRESENCE OR ABSENCE OF X-RADIATION WHEN ADRENAL CORTICAL EXTRACT IS ADMINISTERED OR WITHHELD

Difference between zero hours and:	X-RADIATION			
	ADMINISTERED		WITHHELD	
	Adrenal Cortical Extract		Adrenal Cortical Extract	
	Administered	Withheld	Administered	Withheld
2 hours	+ 1.38	- .75	+ .08	+ .42
4 "	- .08	- 1.12	- 1.04	+ 1.17
6 "	- 1.75	- 1.54	- .30	+ 1.17
8 "	+ .64	- 1.75	- 1.00	+ 1.54
10 "	+ 1.62	- 2.12	+ .20	+ 2.04
12 "	+ .71	- 1.92	+ .08	+ 1.71
14 "	+ .66	- 2.96	+ .16	+ 2.04
18 "	+ 1.20	- 3.96	- .96	+ 1.12
24 "	- 1.96	- 3.25	- .63	+ .21
30 "	- 2.04	- 3.04	- .29	+ .46
48 "	- 4.34	- 4.00	- .08	+ .34
498 "	- 4.29	- 4.29	+ .46	+ .79

Note: See note for table 1.

and the group without x-radiation are broken down separately into two subgroups, namely, those receiving adrenal cortical extract and those not receiving adrenal cortical extract. The most interesting conclusion which may be drawn from this table is that among the animals which received x-radiation, those receiving adrenal cortical extract showed a slight increase in antibody titer through the 18-hour period, whereas those not receiving the adrenal cortical extract showed an almost steady decline in antibody titer through the entire period. The differences between these two groups at the 10-, 12-, and 14-hour periods are significant at the 5 percent level and at the 18-hour period are significant at the 1 percent level. Adrenal cortical extract was administered at the 0-, 6-, 12-, and

30-hour periods. It is clear that after the cessation of the adrenal cortical extract treatment of the animals exposed to x-radiation the antibody content in those receiving adrenal cortical extract dropped rapidly to the level of those which had not been given adrenal cortical extract. Actually, this drop in titer began following the 18-hour period, and the possible explanation for this may be that an interval of 18 hours elapsed between the last two injections of adrenal cortical extract, whereas only 6 hours elapsed between each of the earlier injections. This may account for the failure of the last injection of adrenal cortical extract to produce a significant increase. It may be that the effect occurred between the 30- and 48-hour measurements.

Among the animals not exposed to x-radiation, those in the subgroup which received adrenal cortical extract showed no appreciable change in antibody titer, whereas those in the subgroup which did not receive adrenal cortical extract showed a slight increase in titer during the first 18 hours and then returned to their original level. None of the differences between these subgroups is statistically significant.

Table 3 is like Table 2, except that it compares the results for the group receiving additional antigen with those from the group exposed to the effects of whole body x-radiation. None of the differences among the means in this table is statistically significant. Nevertheless, some interesting behaviors are implied. It appears that among the animals which were exposed to x-radiation, those which received no antigen suffered an immediate and gradually increasing drop in antibody titer, whereas those which received additional antigen maintained titers for an extended period and then dropped rapidly after 48 hours to the level of the group which received no additional antigen. However, another interpretation would be that among those animals which received no additional antigen, those which were exposed to x-radiation showed an immediate and sustained drop in antibody titer, whereas those which did not receive x-radiation maintained their antibody titers throughout the experiment. This interpretation is quite reasonable in the light of the findings of other investigators and may be

preferred to the former. In either situation, it should be remembered that none of these differences is considered statistically significant.

TABLE 3. EXPERIMENT 1

AVERAGE CHANGES IN CIRCULATING ANTIBODY TITERS IN THE PRESENCE OF X-RADIATION WHEN ANTIGEN IS ADMINISTERED OR WITHHELD

Difference between zero hours and:	X-RADIATION			
	ADMINISTERED		WITHHELD	
	Antigen		Antigen	
	Administered	Withheld	Administered	Withheld
2 hours	+ .12	− 2.25	+ .08	+ .42
4 "	+ .71	− 1.92	+ .12	0
6 "	+ .62	− 2.67	+ .29	+ .50
8 "	+ .08	− 1.38	+ .37	+ .17
10 "	+ 1.71	− 2.21	+ 1.12	+ 1.12
12 "	+ .25	− 1.46	+ .96	+ .84
14 "	+ .12	− 2.42	+ 1.21	+ 1.00
18 "	+ .83	− 3.58	− .46	+ .62
24 "	− 1.34	− 3.88	− .84	+ .42
30 "	− 1.25	− 3.83	− .79	+ .96
48 "	− 4.67	− 3.66	− .75	+ 1.17
498 "	− 4.58	− 4.00	− .17	+ 1.42

Note: See note for table 1.

Since the effect of primary interest in this investigation is that of x-radiation on circulating antibodies as modified by the administration of adrenal cortical extract, Table 4 and Figures 1 and 2 were prepared from the results on the eight animals which did not receive additional antigen. It may be seen that, for the most part, the effects manifested in Table 4 and Figures 1 and 2 are the same as the effects observed previously with all sixteen animals.

An attempt was made to simulate some of the conditions in which an immunized animal might be exposed to the ionizing radiation of an atomic explosion and then to ascertain its antibody changes when handled in a variety of ways. In order

TABLE 4. EXPERIMENT 1

AVERAGE CIRCULATING ANTIBODY TITERS OF IMMUNIZED RABBITS IN THE PRESENCE OR ABSENCE OF X-RADIATION AND ADRENAL CORTICAL EXTRACT

SAMPLING PERIOD: Number of hours following initiation of the experiment	CONTROLS				X-RADIATION				X-RADIATION WITH ADRENAL CORTICAL EXTRACT				ADRENAL CORTICAL EXTRACT			
	Replication				Replication				Replication				Replication			
	I		II		I		II		I		II		I		II	
	* Titer	Conc. **	Titer	Conc.	Titer	Conc.	Titer	Conc.	Titer	Conc.	Titer	Conc.	Titer	Conc.	Titer	Conc.
0 hours	11.0	1.00	8.3	1.00	12.3	1.00	11.8	1.00	14.2	1.00	6.5	1.00	12.0	1.00	11.0	1.00
2 "	12.5	1.14	8.3	1.00	8.7	.71	12.0	1.02	8.8	.62	6.3	.97	12.0	1.00	11.2	1.02
4 "	13.0	1.18	8.0	.96	9.0	.73	11.5	.98	10.2	.72	6.5	1.00	10.3	.86	11.0	1.00
6 "	12.7	1.16	8.2	.99	8.3	.68	11.5	.98	8.3	.59	6.0	.92	12.3	1.03	11.2	1.02
8 "	13.3	1.21	8.7	1.05	10.5	.85	10.7	.91	11.3	.80	6.8	1.05	10.7	.89	10.3	.94
10 "	12.8	1.16	10.2	1.23	8.0	.65	10.5	.89	11.0	.78	6.5	1.00	12.3	1.03	11.5	1.05
12 "	12.5	1.14	10.5	1.27	8.5	.69	10.0	.85	12.3	.87	8.2	1.26	12.2	1.02	10.3	.94
14 "	13.2	1.20	10.2	1.23	6.2	.50	8.5	.72	12.0	.85	8.3	1.28	12.3	1.03	10.7	.97
18 "	13.3	1.21	10.7	1.29	6.2	.50	6.5	.55	9.3	.66	8.5	1.31	10.0	.83	10.8	.98
24 "	11.0	1.00	11.2	1.35	6.5	.53	6.0	.51	10.3	.73	6.5	1.00	11.3	.94	10.5	.95
30 "	13.0	1.18	10.8	1.30	6.5	.53	6.5	.55	10.2	.72	6.3	.97	12.3	1.03	10.2	.93
48 "	12.8	1.16	11.2	1.35	6.5	.53	7.0	.59	10.5	.74	6.2	.95	12.7	1.06	10.3	.94
498 "	12.7	1.16	12.0	1.45	6.0	.49	6.3	.53	10.2	.72	6.3	.97	12.5	1.04	11.0	1.00

* Antibody titers = $1:10^3$, i. e., 11.0 = 1:11,000

** Concentration of antibody = The 0 hours antibody titer is considered to be equivalent to a concentration of 1.0. Thus, changes in antibody titers of different sampling periods are expressed as multiples or fractions of the antibody concentration for an animal at the initiation (0 hours) of the experiment.

▼ Period at which adrenal cortical extract was administered.

to eliminate the additional variant of the rapidity with which antibodies to a new antigen are formed, an antigen was employed against which the experimental animals had already been immunized. Effects in the few hours shortly after exposure to x-radiation could be studied with greater ease by working with a previously formed antibody.

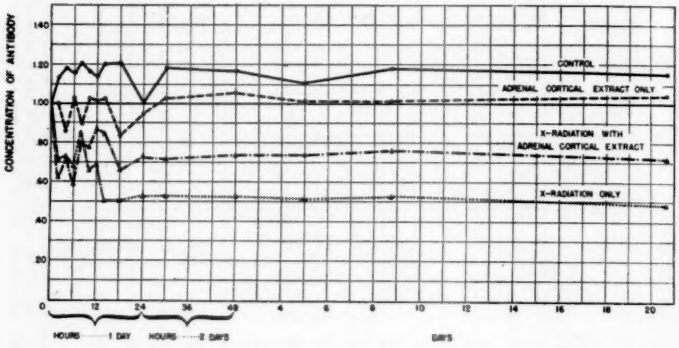

FIG. 1. Circulating Antibody Concentrations, Replication I, Experiment 1.

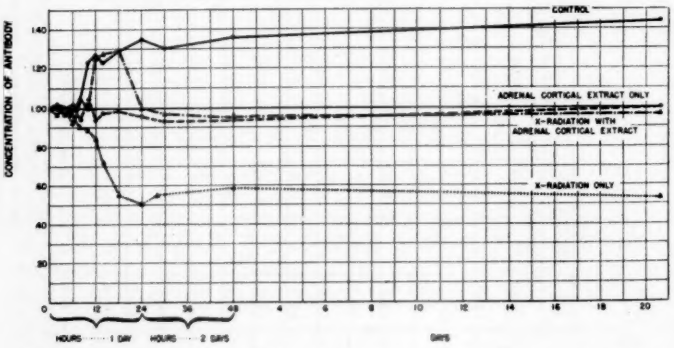

FIG. 2. Circulating Antibody Concentrations, Replication II, Experiment 1.

The data obtained under the conditions of this experiment indicate that there is a possibility of being able to alter the course of antibody response after whole body x-radiation. It was noted that at the 498-hour (21-day) period the groups of rabbits which received adrenal cortical extract showed no

permanent increase in antibody titers above the groups of x-radiated animals which had not received adrenal cortical extract. However, there was a substantially higher titer of circulating antibody at the 18-hour period during the time of actual treatment with adrenal cortical extract.

Experiment Number 2

In this experiment the effects of the absence or presence of each of two treatment factors, namely, x-radiation and adrenal cortical extract, were observed in thirty-eight rabbits which had been immunized to a condition of high agglutinating titers with *Salmonella typhosa* antigen. The last injections of the immunizing antigen were made 6 days before any of the animals was exposed to x-radiation. Circulating antibody titer determinations were made on all animals at eight different time intervals over a 35-day period of observation, starting one day prior to x-radiation and then at intervals of 1, 5, 8, 14, 20, 27 and 34 days following x-radiation.

The circulating agglutinating antibody measurements obtained at eight sampling intervals for each of the experimental animals in the different groups are given in Table 5. For the purpose of analysis the differences in antibody titer measurements between each of the sampling intervals 2 through 8 and the first sampling interval were computed. These are presented in Table 6. The circulating antibody titers are expressed in thousands so that a mean of —1.5 for a particular sampling interval would indicate an average drop in antibody titer of 1,500 from that obtained at sampling interval 1.

The interaction of the effects of x-radiation and adrenal cortical extract was considered to be most important in the second experiment. A study of the results should indicate whether the difference between changes in circulating antibody titers for the x-radiation group and the group which had not been exposed to x-radiation is the same for the group receiving adrenal cortical extract and for the group to which no adrenal cortical extract had been administered. In addition, the results should indicate whether the administration of adrenal cortical extract succeeded in maintaining the circulating antibody titers of the x-radiated animals at a level

significantly higher than the titers of the x-radiated animals which had not received adrenal cortical extract.

Under the conditions of this experiment it was found that the x-radiation and adrenal cortical extract interaction was significant for all sampling periods except the last one. In order to illustrate these effects more clearly the mean differences shown in Table 6 for the four experimental groups are plotted by pairs in Figures 3, 4, 5, and 6. Differences in circulating antibody titers which are significant at the 5 percent level are indicated.

TABLE 5. EXPERIMENT 2

CIRCULATING ANTIBODY TITERS OF IMMUNIZED RABBITS IN THE PRESENCE OR ABSENCE OF X-RADIATION AND ADRENAL CORTICAL EXTRACT

Designation and description	Animal number	ANTIBODY TITERS* AT DIFFERENT SAMPLING INTERVALS							
		1	2	3	4	5	6	7	8
		-1 day	+1 day	+5 days	+8 days	+14 days	+20 days	+27 days	+34 days
(1) Control (No treatment)	1	3.5	3.5	4.0	3.5	3.5	2.5	2.5	2.5
	2	4.0	4.0	4.0	4.0	4.0	3.5	2.5	2.5
	3	3.5	3.5	3.5	3.5	3.0	2.5	2.5	3.0
	4	3.5	3.5	3.5	4.0	3.5	3.0	2.5	2.0
	5	6.0	6.5	6.5	6.5	4.5	4.5	4.0	4.5
	6	4.0	4.0	4.0	3.5	4.5	4.0	3.5	2.5
	7	6.0	5.0	6.5	6.0	6.5	6.5	6.0	5.0
	8	9.0	9.0	9.0	8.5	7.0	7.5	7.0	4.0
	9	6.0	6.5	7.0	6.5	5.5	5.0	5.0	4.0
a. X-radiation only **	10	4.0	3.0	2.0	2.0	2.0	1.5	1.5	2.0
	11	5.0	3.5	3.5	3.0	2.5	2.5	1.5	4.0
	12	6.5	5.5	4.0	3.0	2.5	2.0	2.0	4.0
	13	5.5	4.0	3.5	3.0	2.0	2.5	2.0	4.0
	14	5.0	4.5	4.0	3.0	2.5	dead	dead	dead
	15	9.0	6.0	5.5	3.5	2.5	2.5	2.0	2.5
	16	7.0	5.0	4.0	4.0	dead	dead	dead	dead
	17	8.0	6.0	5.5	5.0	5.5	5.0	3.0	2.5
	18	7.0	5.5	4.5	4.0	4.5	4.5	4.5	5.5
b. Adrenal cortical extract only ***	19	3.0	3.5	3.5	3.0	3.0	2.5	1.5	2.5
	20	2.5	2.5	2.5	2.5	2.0	2.0	1.5	2.0
	21	4.0	4.0	4.0	4.0	3.0	3.0	3.0	3.5
	22	3.0	3.0	3.0	3.0	2.5	2.5	2.0	3.0
	23	5.0	5.5	5.0	5.0	4.0	4.0	3.0	2.5
	24	6.0	6.0	6.0	6.0	6.0	5.0	5.0	3.5
	25	6.0	6.5	6.0	5.5	5.5	4.5	4.0	3.0
	26	10.5	11.0	11.0	10.5	9.5	9.5	8.5	6.5
	27	8.0	9.0	9.0	8.0	7.5	7.0	7.0	5.0
	28	6.5	7.0	6.5	6.5	6.0	5.5	5.5	4.0
ab. X-radiation with adrenal cortical extract ***	29	3.0	3.0	3.0	2.5	2.5	dead	dead	dead
	30	4.5	5.0	4.5	3.5	4.0	3.5	3.5	2.5
	31	5.5	6.5	6.0	5.5	5.0	4.5	3.5	3.5
	32	4.5	4.5	4.5	4.0	4.0	4.0	3.5	2.5
	33	5.5	4.0	4.5	4.0	3.0	dead	dead	dead
	34	5.5	5.5	6.0	5.0	3.5	2.0	2.5	1.0
	35	10.0	10.5	10.5	10.5	9.5	9.5	9.5	8.0
	36	10.5	10.0	10.0	9.5	9.5	9.5	9.5	6.0
	37	10.5	10.5	dead	dead	dead	dead	dead	dead
	38	8.0	8.5	8.0	7.0	7.5	8.0	7.0	3.0

* Antibody titers = 1:10^3, i. e., 3.5 = 1:3,500

** X-radiation exposures done on the day between sampling intervals 1 and 2.

*** Adrenal cortical extract administered as 5. ml. subcutaneously into the skin of the back one hour before x-radiation and 1 ml. each day for the next 14 days.

TABLE 6

Mean Differences in Antibody Titers (Plotted by Pairs of Groups in Figures 3–6) Experiment 2

	BETWEEN SAMPLING PERIODS							
	1 and 1	1 and 2	1 and 3	1 and 4	1 and 5	1 and 6	1 and 7	1 and 8
(1). Control (no treatment)	0	0.11	0.29	0.04	-0.39	-0.70	-1.09	-1.79
a. X-radiation alone	0	-1.52	-2.25	-2.90	-3.25	-3.56	-4.17	-3.12
b. Adrenal cortical extract	0	0.35	0.20	-0.05	-0.55	-0.90	-1.35	-1.90
ab. X-radiation and adrenal cortical extract	0	0.05	0.01	-0.60	-0.95	-1.04	-1.35	-3.00

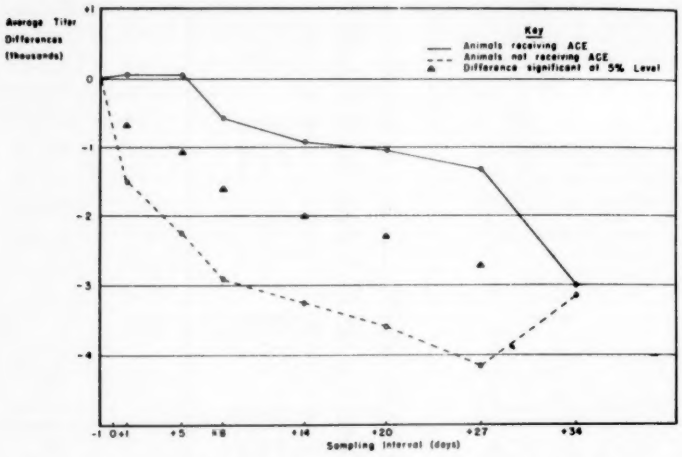

FIG. 3. Average Titer Differences of Animals Receiving X-Radiation.

In this experiment it was possible to alter the course of circulating antibody response following whole body x-radiation. From an examination of the data in the above tables and figures there can be little doubt that under the conditions of this study

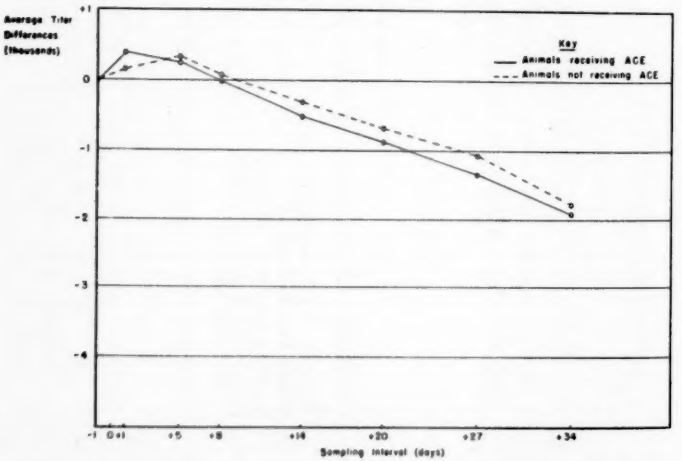

FIG. 4. Average Titer Differences of Animals Not Receiving X-Radiation.

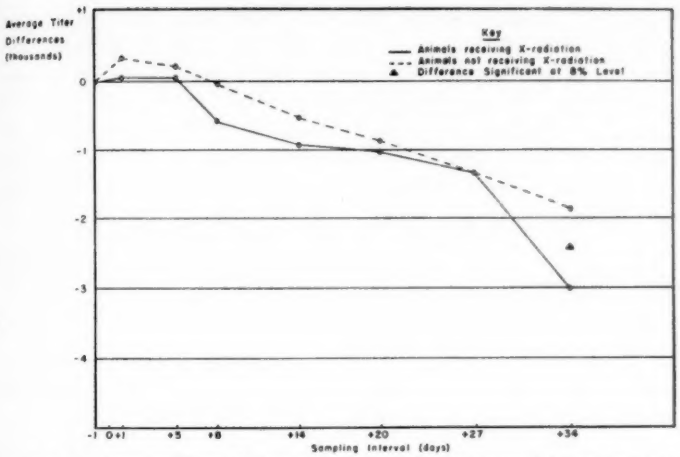

FIG. 5. Average Titer Differences of Animals Receiving Adrenal Cortical Extract.

the administration of adrenal cortical extract succeeded in maintaining the circulating agglutinating antibody titers in the x-radiated rabbits, at least through the 27-day sampling

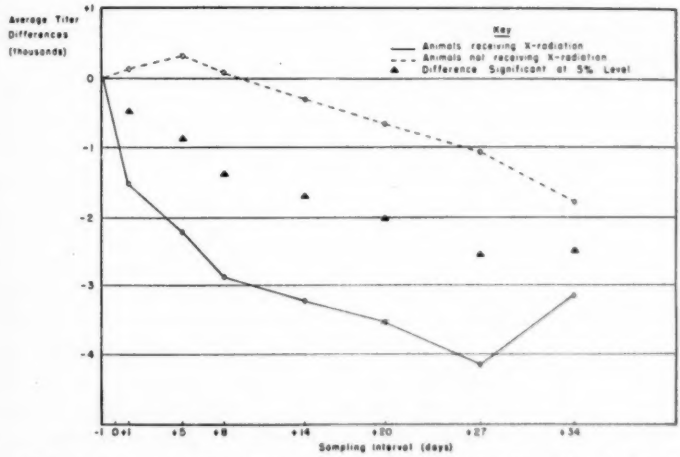

FIG. 6. Average Titer Differences of Animals Not Receiving Adrenal Cortical Extract.

interval. There is some indication that after this period the protective action begins to wear off. However, if the administration of adrenal cortical extract had continued for a longer period the protection might have continued. Further experimental evidence is needed to substantiate these points. It would be interesting to determine the capacity of the animals in the four experimental groups to respond to additional doses of the immunizing antigen at intervals following exposure to x-rays. Such an experiment could be designed to show whether the reticulo-endothelial systems of rabbits within the various experimental groups have protected or enhanced capacities to produce new supplies of circulating antibodies.

The observation that circulating antibody enhancement was obtained with administration of adrenal cortical extract to x-radiated animals is believed to be an important one.

Summary

Studies are being continued for promising lines of research in the regulation of antibody production in animals with severe, whole body ionizing radiation injury. The present work deals with the effects of adrenal cortical extracts as well as stimulating doses of specific antigen upon circulating antibody titers in x-radiated animals previously immunized against *Salmonella typhosa*.

In the first experiment, rabbits which received stimulating doses of antigen during the 21-day observation period responded with slight increases in antibody titers. Those which received no stimulating doses showed no regular antibody changes. Animals receiving adrenal cortical extract at selected intervals following immunization showed no significant changes from controls, whereas those given 500 r whole body x-radiation, without follow-up stimulating antigen, demonstrated prompt and well-defined decreases in circulating antibody titers. The administration of adrenal cortical extract to x-radiated animals resulted in a significant maintenance of antibody titers for most of the treatment period. Similarly, although lacking statistical confirmation, x-radiated animals given a stimulating dose of antigen immediately before x-radiation appeared better able to maintain their antibody titers during the observation

period, as compared with x-radiated animals without the stimulating doses of antigen.

In the second experiment two treatment factors were used in rabbits previously highly immunized against *Salmonella typhosa:* x-radiation and adrenal cortical extract. Repeated post radiation doses of adrenal cortical extract succeeded in maintaining antibody titers significantly higher than in those irradiated animals that did not receive the extract.

The data obtained under the conditions of these experiments indicate that it is possible to alter the usual course of antibody response following whole body ionizing radiation injury and, that in the x-radiated animal, circulating antibody enhancement may be produced by giving adrenal cortical extract during the post radiation period.

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SERUM ANTIDIURETIC ACTIVITY IN ESSENTIAL HYPERTENSION*

L. Miller De Paiva, Olga B. Henriques, and S. B. Henriques

The antidiuretic activity of human serum has been investigated in various clinical conditions accompanied by changes in water metabolism, such as eclampsia (1), pregnancy (2) and hypertension (3).

The experimental production of hypertension by administration of posterior pituitary preparations indicates the possibility of this gland being one of the many causes of human hypertension. Since the neurohypophysis is under the direct influence of hypothalamic centers, we thought that differences in serum antidiuretic activity could be detected between the neurogenic and established phases of essential hypertension.

The experiments reported in this paper were undertaken in order to compare the serum antidiuretic activity of patients in those phases of essential hypertension.

Material and Methods

Sera of 16 women with "neurogenic"[1] hypertension and 12 patients with established essential hypertension, associated or not to different diseases were used in this study. As controls the sera of 14 subjects with normal blood pressure and in which no disease was detected were used.

After being collected the sera were kept frozen in a refrigerator at —10° C till the moment of the test when they were heated to room temperature. The period of storage varied from 1 to 10 days.

The method of determining the antidiuretic activity was that of Pendergrass, Hodes and Griffith (1941), with the difference that the rats were fasted only for 6 hours before the test. For each serum 3 rats of 200–300 gm. were used. These were kept together in a metabolism cage during the test.

*From the Escola Paulista de Medicina, Laboratórios de Farmacologia e Bioquímica, São Paulo, Brazil. Received for publication March 18, 1951.

[1]The neurogenic phase of essential hypertension (9, 10) was diagnosed by psycho-analysis, hyperpnea (11) and sodium amytal tests. In this paper this phase of the disease will be called "neurogenic" hypertension.

Our working conditions permitted only 8 tests to be performed each time. The modern design of experiments would require that the different classes of sera would be equally distributed in each determination. This was not observed as it was impossible to obtain at the same time equal numbers of sera from patients with hypertension and normal subjects; but with each series of determinations one test with saline was run.

The rats were repeatedly used with a minimum interval of 72 hours.

Results

Tables I to III show all the data pertinent to the patients examined. It can be seen, as indicated by the column of "controls" of any table, that a determination with 0.9% sodium chloride was always run together with every serum. This was done as a check for the possible fluctuation of the sensitivity of the rats throughout the whole experiment.

It can be seen that the average water retention caused by saline varied slightly: from 1.4 (table II) to 1.9 (table III). Statistical analysis of the results failed to detect correlation between the water retention of the "controls" and that of the serum treated animals. Moreover, Table I shows that the water retention caused by injection of normal serum is significantly greater than that caused by injection of saline.

TABLE I

The Antidiuretic Activity of the Serum of Normal Subjects

Subject	Age	Sex	Blood pressure	Water retention per 100 gm. rat	Water retention per 100 gm. rat *Controls**
				ml	ml
A.G.	24	F	120–80	1.9	1.6
E.M.C.	26	F	120–80	1.9	1.2
L.W.	24	F	120–80	1.7	1.4
L.B.G.	27	F	120–80	2.3	1.6
M.G.	20	M	110–85	2.5	1.9
M.H.F.	20	F	110–80	2.4	1.6
O.D.	23	F	120–75	2.2	0.9
R.S.F.	19	F	120–80	2.4	1.4
W.F.	21	F	120–80	1.6	1.6
R.C.	18	F	115–80	2.7	2.9
E.P.	19	F	125–80	4.5	2.9
M.E.P.	25	F	120–80	2.7	1.0
C.M.	22	M	125–85	2.3	1.3
			Averages and standard errors	2.4 ± 0.20	1.6 ± 0.17

*Injected with 1 ml. saline.

TABLE II

The Antidiuretic Activity of the Sera of Patients with "Neurogenic" Hypertension

Patients	Age	Blood pressure mm Hg Control period	Blood pressure mm Hg After 1 min. hyperpnea	Water retention/ 100 gm. rat ml	Water retention/ 100 gm. rat Controls* ml	Remarks
J.P.M.	43	160/100	140/95	3.6	1.0	.....
Z.S.	45	170/90	150/90	3.0	1.4	.....
Z.B.M.	48	185/100	160/100	4.1	1.8	Menopause
M.L.F.	46	200/100	160/90	4.2	1.0	Menopause
M.A.V.	49	180/90	150/90	2.9	1.5	Menopause
R.F.	54	170/100	140/90	3.0	1.6	Menopause
C.M.	52	180/100	150/95	2.5	1.0	Menopause
C.G.B.	49	190/110	145/90	3.3	1.5	Menopause
E.C.F.	56	180/90	150/90	4.6	1.5	Obesity
L.R.M.	47	190/100	170/95	2.7	1.6	Obesity
M.M.	32	170/90	140/90	2.0	1.3	Obesity
A.S.	43	180/100	150/90	3.3	1.3	Obesity
C.M.L.	49	210/100	160/90	3.6	1.4	Menopause
M.S.	52	230/120	190/110	2.6	1.6	cervix carcinoma (opt)
N.C.	35	150/90	125/85	3.2	1.4	Menstrual tension
P.G.	41	160/100	140/90	2.6	1.4	Menopause
Averages and standard errors				3.2 ± 0.17	1.4 ± 0.06	

*Injected with 1 ml. saline.

Therefore, it is obvious that it is useful to compare only the average water retention caused by normal serum with that caused by hypertensive sera.

TABLE III

The Antidiuretic Activity of the Sera of Patients with Established Essential Hypertension

Patients	Age	Sex	Blood pressure mm Hg Control period	Blood pressure mm Hg After 1 min. hyperpnea	Water retention/ 100 gm. rat ml	Water retention/ 100 gm. rat Controls* ml	Remarks
J.S.	56	F	200/100	190/105	3.4	1.6	Obesity
L.D.	41	F	160/90	155/90	4.2	1.6	Obesity
M.T.	47	F	160/80	145/85	3.6	2.9	Obesity
A.L.B.	45	F	170/110	170/110	3.7	3.0	Obesity
C.M.V	29	F	230/140	230/140	3.4	1.9	Lues
M.L.S.	35	F	160/100	160/100	3.2	1.4	Pregnancy
M.J.P.	49	F	180/100	170/95	2.6	1.0	Menopause
T.P.S.	62	F	200/90	200/90	4.1	2.9	Diabetes
V.A.	58	M	150/100	140/95	3.7	1.0	Climaterium
J.A.F.	54	M	180/120	180/120	4.3	3.0	Climaterium
L.P.C.	43	F	170/100	165/100	2.7	1.3	.....
C.R.S.	29	F	170/100	165/100	3.7	1.3	.....
Averages and standard errors					3.55 ± 0.15	1.9 ± 0.23	

*Injected with 1 ml. saline.

Perusal of Tables I, II and III shows that while normal serum caused a water retention ranging from 1.7 to 4.5 (average 2.4) with only one case *above* 2.7, neurogenic hypertensive serum caused a retention from 2.0 to 4.6 (average 3.2) with only 3 values *below* 2.7. Established essential hypertension produced a retention of 2.6 to 4.3 (average 3.5) with only one value *below* 2.7.

The *t* test analysis revealed a highly significant difference in favor of the hypertensive patients as compared with the normal subjects, while no significant difference is observed between the two groups of hypertensives. This is better seen by inspection of Chart I.

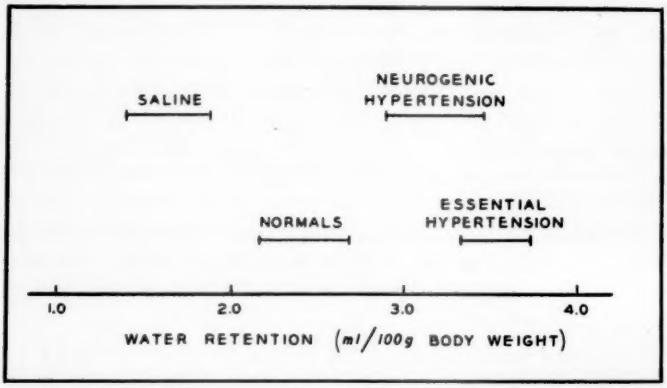

CHART 1. Water retention of rats treated intraperitonally with saline, sera of normal subjects and of patients with essential and "neurogenic" hypertension. The range indicated by the linea was a probability of 80%.

Inspection of Tables II and III seems to indicate also that the associated clinical conditions do not influence the antidiuretic activity of the serum of hypertensive patients.

Discussion

The data of the literature leave little room for doubt on the presence of antidiuretic activity in the blood (Pendergrass, et al., 1941) and urine (Ellis and Grollman, 1949) of hypertensive patients. However, the nature and origin of this activity is still obscure. The evidences presented by Pendergrass, et al. (1941),

for the neurohypophyseal origin of the antidiuretic substance of hypertensive patient's serum are unconvincing, and in the case of eclampsia this origin has been doubted (Shaffer, Cadden, and Stander, 1941). On the other hand Birnie and associates (1949), have presented evidence for the hypophyseal origin of the antidiuretic substance of rat's serum. Yet it remains to be proved a common origin for the rat and human serum antidiuretic activity.

In confirmation of other authors (Pendergrass, et al., 1941) we observed a high antidiuretic activity in most of the hypertensive sera studied. Our results suggest that this activity is independent of the neurogenic character of the disease.

We observed also the presence of antidiuretic activity in normal sera; this confirms Lloyd and Lobotsky (1950), and is in disagreement with Griffith and coworkers (1942).

The inclusion in Table II of a patient with premenstrual tension is due to the fact that in contraposition to Lloyd and Lobotsky (1950), we did not observe (Miller De Paiva, O. B. Henriques, and S. B. Henriques), any increase of antidiuretic activity in this clinical condition except when associated to nervousism.

Summary

The serum antidiuretic activity of normal subjects and patients with essential hypertension in the neurogenic and established phases of the disease was studied.

Antidiuretic activity was observed in normal serum as compared to saline. The water retention caused by the sera of the patients with essential hypertension was significantly higher than that produced by normal sera, independently of the neurogenic character of the disease.

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INFLUENCE OF GENERAL HYPOXIA ON LOCAL COLD INJURY*

JOSEF PICHOTKA, ROBERT B. LEWIS, AND ULRICH C. LUFT

Observations on frostbite suffered in aircraft (Davis, Scarff, Rogers, and Dickinson, 1943) and on mountains (Greene, 1941; Luft, 1942) indicate that the incidence and severity of local cold injury are greater at high altitudes than at sea level. Considering the circumstances under which these impressions were gained, it is a matter of conjecture whether low temperatures accompanied by high wind velocities were mainly responsible for the aggravation of frostbite, or if it was due to the combination with general hypoxia. The latter possibility seemed worthy of further investigation under controlled conditions on animals.

The question arises to what extent a state of oxygen deficiency predisposes toward cold lesions and whether it affects the recovery process following the exposure. Three sets of experimental conditions readily suggested themselves from this aspect: first, the effect of hypoxia coincident with and limited to the period of exposure to cold; second, cold exposure at the end of a prolonged period of oxygen deficiency; and finally, the combination of cold injury and hypoxia maintained after exposure to cold.

Material and Method

The experiments were performed on male albino rabbits of more than 2,500 grams body weight which were permanently kept at 25° C. and fed a standard diet (Purina). One hundred and thirty-two animals were exposed to cold injury and hypoxia; during the same period 60 animals were subjected to a standard cold injury and used as controls. Two different standard cold injuries were used; in one, a hind leg was immersed for 30 minutes in an alcohol bath of —12° C. and in the other, the temperature was —15° C. The exact procedure is described in another paper (Pichotka and Lewis, 1949).

The autopsies were performed on the seventh or eighth day after exposure. At this time the superficial (skin) as well as

*From the Department of Pathology, USAF School of Aviation Medicine, Randolph Field, Texas. Received for publication March 26, 1951.

the deep (muscle) necrosis is usually sharply demarcated. The necrotic area in the muscle protrudes and is indurated. The border between healthy and necrotic muscle tissue is often emphasized by a small hemorrhagic zone.

The extent of injury was determined independently as superficial necrosis, pertaining to the skin and subcutaneous tissues only, and as necrosis of the muscles of the exposed leg. In order to avoid alterations in the determination of the superficial necrosis by different degrees of swelling, the borderlines of the necrotic area were transferred to the clean-shaven healthy leg by parallel projection. Ankle and knee joint were taken as fixed points. The area was then transferred from the healthy leg to superimposed onion skin paper and measured with the planimeter.

The determined area of necrosis refers only to the leg between ankle and knee joint. When the necrotic area in this part passes a certain extent the whole foot becomes necrotic. According to our experience, the relations are better represented by not considering the foot and by assuming that the necrosis of the foot is a secondary phenomenon due to extensive necrosis of the leg.

The extent of muscle necrosis was determined by weight. After the leg was skinned down to the ankle joint, four muscles or muscle groups, (1) the musculus tibialis anticus plus musculus extensor digitorum longus, (2) the triceps surae (musculus soleus plus musculus gastrocnemius), (3) the musculus flexor digitorum longus, and (4) the musculus peronaei—longus and brevis, were separately prepared and weighed. The necrotic area, which at this time was usually sharply demarcated, was then carefully cut off with a pair of small scissors. The remaining part of healthy tissue was weighed again and thus the proportion of necrotic tissue determined.

In all experiments hypoxia was produced by transferring the animals to a simulated altitude of 20,00 feet (6,100 m) in the low pressure chamber. The hypoxia corresponding to this altitude (barometric pressure 349 mm Hg) is dangerous for human beings, but has less effect on the rabbit. Rabbits usually can stand such a diminution of the oxygen pressure for hours and days without serious consequences.

The average results are usually given with the standard deviation of the mean. The statistical method used in the computation of some of our data will be discussed later. It should be mentioned that 16 animals appear twice and under different aspects in the tables of this paper.

Results

1. *The influence of hypoxia during exposure to cold injury.* In this group 16 animals were taken to altitude where one hind limb was exposed to —12° C. for 30 minutes after a preparatory period at the final altitude of not more than half an hour. All animals were returned to ground level immediately after the cold exposure. Compared with 11 animals which were subjected to the identical procedure at normal ambient pressure on the same day, no evidence was present in this group that the brief period of hypoxia during the local application of cold had any influence on the extent of the resulting muscle necrosis.

Certain differences in the outward appearance and the consistency of the afflicted limbs between the altitude animals and the controls observed during recovery will be referred to later.

TABLE 1

EFFECT OF HYPOXIA DURING EXPOSURE TO -12°C. FOR 30 MINUTES

CONDITIONS	NO. OF ANIMALS	MUSCLE NECROSIS IN PER CENT	SKIN NECROSIS IN Cm^2
EXPOSURE TO COLD INJURY AND SIMULTANEOUS HYPOXIA	16	26 ± 4.8	0
EXPOSURE TO COLD INJURY ALONE	11	29 ± 4.5	0

2. *The influence of hypoxia during and after exposure to cold injury.* In this group the animals were taken to altitude and immediately exposed to cold injury. They remained under hypoxia for periods varying from 4 to 12 hours following the exposure and were then transferred to normal atmospheric conditions. Forty-eight animals were exposed to cold injury and hypoxia (6 died and 4 did not become frostbitten); 24 control animals were simultaneously exposed to cold injury (1 did not become frostbitten). The results are shown in Table 2.

It occurred in a few cases in both groups that the legs did not solidify during exposure to the cold bath. These cases usually do not develop any necrosis of the exposed tissue. The additional hypoxic period did not change this result. For the estimation of the influence of a period of hypoxia subsequent to exposure to cold injury, these cases are without significance and therefore omitted in the computation.

TABLE 2
THE EFFECT OF HYPOXIA DURING AND AFTER EXPOSURE TO −12°C. FOR 30 MINUTES

DURATION OF HYPOXIA (HOURS)	NO. OF ANIMALS	MUSCLE NECROSIS IN PER CENT	SUPERFICIAL NECROSIS NO. OF CASES	DEATHS
0	23	34.1±3.9	1	0
4 − 6	10	47.8±5.6	4	
6 − 8	8 } 38	53.0±6.1 } 54.6±2.8	3 } 16	6
10 − 12	20	59.1±3.1	9	

Thirty-eight animals exposed to cold injury and hypoxia lost on the average 55 ± 2.8 per cent of the muscle tissue of the exposed leg by necrosis. The corresponding figure for 23 animals simultaneously exposed to the same cold injury is 34 ± 3.9 per cent. The difference is statistically highly significant ($t = 4.7$, $n = 59$, $P < 0.001$). The influence of hypoxia is well illustrated when the extent of necrosis is calculated with regard to the increasing period of oxygen deficiency. The amount of necrotic tissue increases from 34 per cent for animals exposed to cold injury only to 48 per cent with 4 to 6 hours additional hypoxia, to 53 per cent with 6 to 8 hours, and 59 per cent with 10 to 12 hours subsequent hypoxia.

The influence of hypoxia on the extent of injury is also shown by the distribution of cases with superficial necrosis. Of 24 controls with cold injury, only one case resulted in superficial necrosis, while out of 42 animals exposed to cold injury and hypoxia, 16 showed superficial necrosis. The probability of this distribution occurring by chance is less than one per cent ($P = 0.006$).

The results of this group demonstrate clearly that hypoxia following cold injury increases the extent of muscle necrosis and the occurrence of surface necrosis. Under the conditions of our experiments, the amount of damage showed a direct relationship to the duration of the hypoxic period.

3. *The influence of pre-existing hypoxia on local cold injury.* In this group the animals were taken to altitude and exposed to cold injury after being under hypoxia for periods of different length. The hypoxic period ended immediately after the exposure to cold injury. In order to obtain greater differentiation the experiments were performed with two different degrees of standard cold injury. Sixty animals were exposed to —12° C. for 30 minutes and the extent of muscle necrosis was determined; 24 animals were exposed to —15° C. and the occurrence and the extent of surface necrosis were estimated. The results of both groups were compared to simultaneous control groups. The hypoxic period preceding the cold injury varied from 0 to 5 hours.

In 57 animals which survived the experiment the average loss of muscle tissue by necrosis amounted to 26 ± 2.3 per cent. This value is slightly lower than that for control animals without a period of hypoxia preceding the cold injury; but the difference is not significant. The picture changes when the results are arranged according to the duration of the hypoxic period, as is done in Table 3.

TABLE 3

EXTENT OF MUSCLE NECROSIS BEFORE AND DURING EXPOSURE TO —12 °C FOR 30 MINUTES

GROUP	A		B	C			D
	1	2	3	4	5	6	7
DURATION OF HYPOXIA — Hrs	0 — .5	.5 — 1	1 — 1.5	1.5 — 2	2 — 2.5	2.5 — 3	3 — 5
NUMBER OF CASES	8	8	8	8	6	8	11
AVERAGE MUSCLE NECROSIS IN PER CENT	24	29	43	25	25	24	14

MEAN VALUES: 35 ANIMALS EXPOSED TO COLD INJURY ONLY = 31.1 ± 3.1 %
57 ANIMALS EXPOSED TO HYPOXIA AND COLD = 26.0 ± 2.3 %
GROUP A = 27 ± 4.8 %
GROUP B = 43 ± 5.4 %
GROUP C = 25 ± 3.4 %
GROUP D = 14 ± 2.0 %

Each of the groups comprises the animals with the same length of the hypoxic period prior to the cold injury. Groups 1 to 6 represent increments of ½ hour each; Group 7 consists of the animals with a period of hypoxia from 3 to 5 hours.

The average values of these groups are different. While Groups 1 and 2 and also Groups 4, 5, and 6 are on the same level of average values as the control animals, this level is by

far exceeded by Group 3. Group 7 finally has an average value which is far below that of the controls.

An interpretation of this result, of course, depends on the significance of the differences between the mean values. Since our observations of "per cent necrosis" do not show a normal distribution (Pichotka and Lewis, 1949) a non-parametric test of significance was applied. The only requirement for such a test is that the variable be continuous. The particular test used here (Pitman, 1937) provides for the calculation of a statistic which is distributed approximately as Student's "t". For the purpose of this test the values were rearranged. Group A covers the hypoxic period from 0 to 1 hour, Group B from 1 to 1½ hours, Group C from 1½ to 3 hours, and Group D contains the cases with a hypoxic period from 3 to 5 hours. The results are given in Table 4.

The decisive point in the results is that Group B differs significantly from Group C ($P = 0.01$). That means that the decrease in the extent of necrosis observed in our experiments when the hypoxic period prior to cold injury exceeded 1½ hours can be considered real. The further decrease in the extent of necrosis with hypoxic periods of more than 3 hours, in comparison to the preceding Group C (hypoxic period 1½ to 3 hours), is in this test significant on the 5 per cent level ($P = 0.048$). If we compare Groups C and D to the mean value of the control animals (Table 3), it becomes obvious that Group C is not different, but that the extent of necrosis in Group D is significantly less than in the control animals ($t = 3.1$, $n = 44$, $P = 0.001$).

Less clear is the relation between Groups A and B. The difference between the two groups is not significant but nearly so ($P = 0.07$). Considering all factors, however, we feel justified in assuming that this difference is real.

The effect of pre-existing general hypoxia on the resulting necrosis after a standard cold injury is evidently no simple function. A relatively short period of moderate hypoxia (0 to 1 hour at 20,000 feet) did not influence the result. A hypoxic period of slightly more (1 to 1½ hours) resulted in a definite increase of the average extent of final necrosis. If, on the other hand, the hypoxic periods exceeded 1½ hours the average

extent of necrosis was diminished again. Animals exposed to cold after more than 3 hours of hypoxia displayed a further decline in cold injury, which on the average amounted to only 50 per cent of the necrosis observed in the controls.

Table 4

Comparison of the means by a non-parametric test

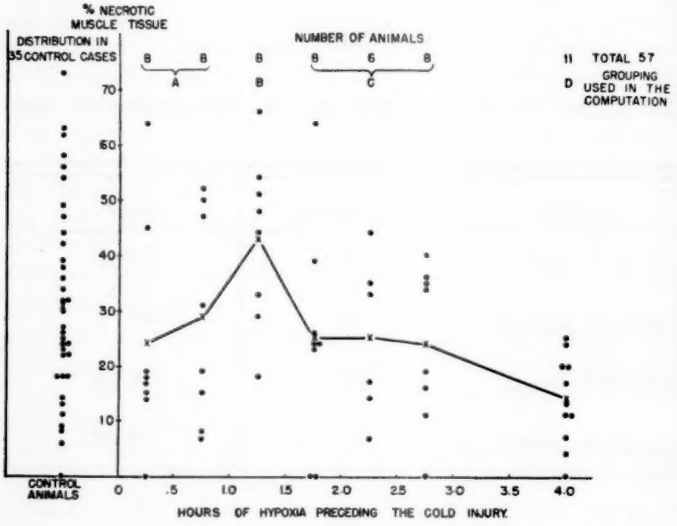
Group	No. in group	Mean
A (0-1 hour)	16	27.50
B (1-1.5 hours)	8	42.75
C (1.5-3 hours)	22	24.59
D (3-5 hours)	11	13.82

Groups compared	"t"	d.f.	P
A and B	1.92	22	0.071
B and C	2.75	28	0.010
C and D	2.07	31	0.048

This behavior is graphically represented in Figure 1. It is obvious from this figure that our observations of "per cent necrosis" do not show a normal distribution but split into a group of lower and a group of higher values. The changes in the average values are mostly due to changes in the distribution between these two groups. The values of the higher level

gradually disappear in the group with more than 1½ hours of hypoxia preceding the cold injury.

FIGURE 1



The same relation as between the extent of muscle necrosis and the length of the hypoxic period prior to exposure exists for the superficial necrosis. In Table 4 the extent of superficial necrosis after exposure to —15° C. for 30 minutes is shown in the same way as it was previously done for the muscle necrosis (Table 5). The overall average for the extent of surface necrosis for animals exposed to cold injury and hypoxia is 14.5 ± 3.4 cm.^{2}, and for animals exposed to cold injury only it is 19.4 ± 4 cm.^{2} These values are not different statistically. But if the results are again considered with increasing length of the hypoxic period prior to the exposure to cold, we arrive at a similar picture as for the muscle necrosis. Animals exposed after a hypoxic period of 1 to 1½ hours again show the most extensive damage. Shorter or longer hypoxic periods are obviously harmful.

Although the data presented in Table 5 do not in themselves justify definite conclusions, they do add considerable support to

the results obtained in the relation of muscle necrosis and duration of hypoxia prior to the cold exposure. In both criteria the trend is strikingly parallel.

Table 5

Extent of superficial necrosis with hypoxia before & after exposure to −15°C. for 30 min.

Duration of hypoxia prior to cold injury	0-.5	.5-1	1-1.5	1.5-2	2-2.5	2.5-3
Superficial necrosis in cm^2	10	30	39	19	14	0
	0	21	62	14	0	8
	17	0	16	26	41	5
	2	0	3			
		0				
		0				
Averages	7.3	8.5	30.0	19.7	18.3	4.3

The average extent of the superficial necrosis for 23 animals exposed to cold injury and hypoxia = $14.5 \pm 3.4\ cm^2$

The average for 10 control animals exposed to cold injury only = $19.4 \pm 4.0\ cm^2$

General Observations

During the first few days after the experiments it was regularly noted that in the animals exposed to cold and hypoxia, whether before or afterwards, the exposed area appeared to be much more severely afflicted than in the corresponding animals which had suffered from cold only. The exposed leg was regularly more swollen and more deeply discolored. But the regression of these changes was also much more pronounced, especially for animals with a hypoxic period prior to cold injury. Despite the worse clinical appearance in the initial phases, the final result in these latter animals was sometimes distinctly better than in the controls.

Discussion

Under our experimental conditions, hypoxia limited to the time of exposure to cold was without any measurable effect on the resulting injury. It appears doubtful whether this holds

true for conditions with less intense heat loss and correspondingly longer times of exposure. The longer the exposure time the more decisive is the role of the peripheral circulation, which at least under certain conditions can be impaired by hypoxia. Furthermore, it has to be taken into account that, with our method of cooling, the exposed tissue was within a few minutes at a temperature range where no measurable metabolism takes place.

Hypoxia following the exposure to a severe cold injury obviously increased the extent of tissue loss considerably. This increase was in direct proportion to the duration of the hypoxic period. In our results the percentage of loss of muscle tissue rose from 33 ± 3 per cent, for animals exposed to the standard cold injury only, to 47 per cent with additional 4–6 hours, to 53 per cent with 6–8 hours, and to 59 per cent with 10–12 hours of subsequent moderate hypoxia. The mechanism suggested by these relations is that an increasing amount of tissue, not yet damaged beyond repair by the cold, is additionally and irreparably injured during the period of general hypoxia. However, on the evidence presented here it cannot be decided whether prolonged local ischemia based upon general hypoxia, or local oxygen deficiency due to low oxygen tension of the blood, or still another factor is mainly responsible for the greater damage during recovery at altitude.

More complicated are the results when a hypoxic period precedes the cold injury. We can derive with certainty from our results that there is no steady increase in the extent of injury with increasing length of the hypoxic period as is the case for hypoxia following cold injury.

Hypoxic periods up to 1 hour did not essentially influence the reaction to a standard cold injury, but hypoxia lasting from 1 to 1½ hours increased the average tissue loss in subsequent cold injury considerably. Longer periods of hypoxia were followed by a gradual decrease in susceptibility to severe cold injury. If hypoxia was maintained for 3 or 4 hours before exposure to cold, the resulting permanent injury was less than under normal atmospheric conditions.

From this it would appear that a critical period occurs in the course of exposure to hypoxia in which the extremities are particularly susceptible to severe cold injury. It is probable that

in this phase cold injury may occur under conditions of heat loss which are ordinarily not injurious. This critical period and the following phase of greater resistance to cold injury found after hypoxia of longer duration are most likely to be the results of adjustments in the course of accommodation to general hypoxia of the organism, which are reflected in the mechanisms of peripheral heat regulation.

It should be mentioned that the significance of the results based on the determination of the extent of muscle necrosis is much better than expressed by the figures in this paper. The percentages given refer to the weight of all leg muscles. But not all muscles are equally susceptible to cold. The musculus tibialis anticus plus the musculus extensor digitorum longus, which weigh about 20 to 25 per cent of the total, are much more prone to cold injury than the other muscles. Necrosis up to 20 per cent usually refers only to these muscles. That means that the extent of muscle necrosis in the different groups would in some regard be more comparable if these 20 per cent were deducted. Such a procedure would, of course, increase the significance of our results.

Conclusions

Hypoxia did not influence the extent of necrosis if present only during the exposure and in a preparatory period up to 30 minutes.

Hypoxia after local cold injury increased the extent of resulting damage as measured by the proportion of necrotic muscle tissue in the exposed leg; the increase was in direct relationship to the period of hypoxia. It is conceivable that general hypoxia induces this effect either by local oxygen deficiency or by impairment of the peripheral circulation.

Hypoxia preceding cold injury influenced the outcome in a complicated manner. Cases with a preparatory hypoxic period from 0 to 1 hour were not different in their results from controls exposed to cold injury only. Slightly longer hypoxic periods (1 to 1½ hours or 1 to 2 hours) resulted in a marked increase in the extent of injury measured as proportion of necrotic muscle tissue and as extent of the superficial necrosis. A further prolongation of the preceding hypoxic period (2 to 3 hours) brought the extent of injury back to that of the controls,

and finally, with a duration of 3 to 5 hours, below that of the controls. It is unlikely that this effect is the reflection of the diminution of the local oxygen tension at the time of exposure. The differences in the extent of necrosis observed after different lengths of the hypoxic period prior to cold injury must therefore be attributed to other factors secondary to general hypoxia.

ACKNOWLEDGMENT

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SEQUENCE OF INCREASING LOCAL COLD INJURY*

JOSEF PICHOTKA, ROBERT B. LEWIS, AND ELLA FREYTAG

Sequence of Increasing Local Cold Injury

Much attention has been focused during recent years on the investigation of local cold injury. A considerable number of experimental papers on this subject have been published, chiefly concerning therapeutic possibilities after establishing what the authors term a "standard cold injury." A more basic approach has been attempted by only a few investigators, and many important aspects of this problem have not been explored. This paper is intended to furnish some of the necessary fundamental knowledge on this subject. The basic purpose of the investigation is to determine the temperatures occurring in rabbit legs under different standardized conditions of exposure and to observe the ensuing clinical and anatomical changes.

Materials and Methods

The experiments were performed on 438 male albino rabbits of more than 2,500 gm. body weight. The animals were kept in the open air throughout the year. Usually they were transferred to the air-conditioned animal room 2 weeks before the experiments and returned there after exposure. They were fed Purina rabbit pellets and, occasionally, carrots and had free access to water.

One hind leg was depilated with Zip depilatory cream, coated with a thin layer of wool fat, covered with a snugly fitting rubber condom boot, and exposed to cold in alcohol cooled with carbon dioxide ice. The alcohol bath consisted of a double-walled and wood-insulated container equipped with an agitator so that continuous movement of the cooling fluid was maintained. The desired temperature was kept within $\pm$ 0.5° C. by adding small pieces of dry ice. The animals were tied by three legs to flat lead-weighted boards in such a manner that a hind leg hung down over one edge. These boards

*From the Department of Pathology, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas. Received for publication April 15, 1951.

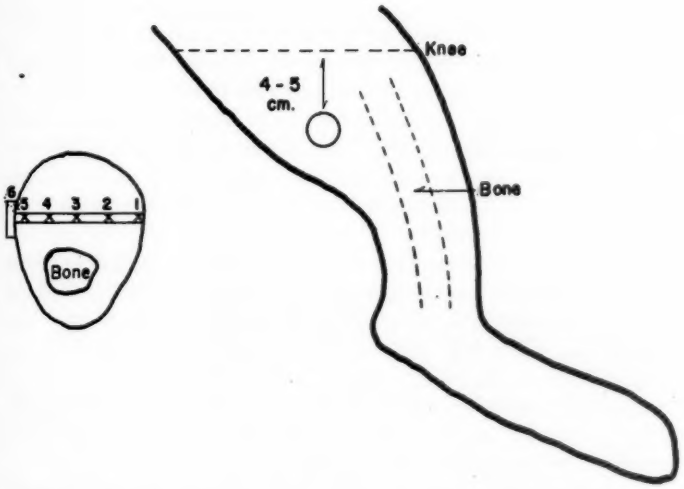
had the advantage that they could be placed in any position, and even struggling animals had difficulty moving them because of their weight. In order to minimize struggling during the exposure, the animals were further fixed to the whole length of the board by wrapping 4-inch roller gauze around animal and board. The dependent leg was immersed in the bath exactly to the knee joint which was marked by a line on the leg. It was kept in this position by a cord fixed to the foot with a strip of adhesive tape and pulled through a ring fastened to the bottom of the bath. Anesthesia was not used.

The time of exposure was 30 minutes in all experiments. After this time the exposed leg was covered with 3 per cent sulfamylon ointment and a sterile dressing applied. The animals were then placed in a cage and left to spontaneous thawing at room temperature (25° C.). Different degrees of cold injury were produced by exposing the legs to different temperatures. The bath temperature was the only variable factor important for the heat transfer in our experiments. Time of exposure, conditions of heat conductivity, and the relative surface of the immersed part of the leg were kept as constant as possible. The range of bath temperatures investigated under these conditions was from + 5° to — 40° C. with 5° intervals. Since it became evident in this investigation that under the described conditions the range from — 10° to — 15° C. was most important for some problems, this interval was split and another group of animals exposed to — 12° C.

For the measurement of the temperatures inside the leg, a special device was developed. A set of 6 thermocouples (copper-constantan) were fitted into a 22 mm. length of ureteral catheter which had five holes at the proper places for the thermocouples. The fine tips of the couples which were distributed over the length of the catheter, as shown in Figure 1, were bent into these holes and carefully fixed flush with the surface of the catheter. A small ivory base plate, fixed to one end, housed the thermocouple for measuring surface skin temperatures. Four holes were drilled in this plate so that it could be sewed to the skin. The whole unit was then covered with two thin layers of insulating lacquer. All wires from the couples were carefully and individually insulated and spun

together to form a common watertight lead. The couples were electrically completely insulated from each other. The heat conductivity in the measuring unit was not higher than in the surrounding tissue, so a thermal influence between the couples could be neglected. Polarizing effects were not possible since the measuring units and all leads were under a watertight cover.

FIGURE 1. Location and arrangement of thermocouples.



This measuring unit was placed through the whole diameter of the leg as follows. A small incision was made through the skin with a sharp scalpel in the middle of the lateral aspect of the leg 4 to 5 cm. below the knee. From this opening a fine channel for the semirigid measuring unit was prepared with the help of a blunt probe. The unit was then placed in position and the base plate sewed to the skin. The common lead from the couples was also sewed to the skin in several places above the immersion line. By this arrangement the measuring unit was sufficiently anchored so that it remained in its exact position throughout the experiment.

When the unit was properly placed, couple 1 at the tip was just beneath the skin and close to the large vein on the medial aspect of the leg. The following three couples (2, 3, and 4) were deep in the muscles of the leg, number 3 being approximately in the center just behind the tibia. The last two couples of the unit were 2 mm. under the skin (5) and on the surface of the skin (6). This latter couple was covered by the rim of the base plate so that no direct contact with the cooling fluid occurred.

The purpose of the temperature measurements with the above device was to establish the typical temperature ranges in the leg during exposure to our standard injuries and also to obtain exact information on the temperature distribution inside the leg. The results are given only as far as they are necessary for the understanding of these points.

Muscle and skin temperatures were determined in 72 animals during and immediately after exposure to various degrees of cold injury. Since all temperature curves for each exposure level were similar, only the curves of 2 or 3 animals were used for the respective graphs.

It soon became apparent that muscle necrosis in the exposed leg preceded the appearance of skin necrosis. The extent of muscle necrosis and its relation to the appearance and the degree of superficial necrosis were therefore determined in 176 animals at the — 12° and — 15° C. level.

Autopsies were usually performed on the seventh or eighth day after exposure, since the necrosis was fully developed and sharply demarcated at that time.

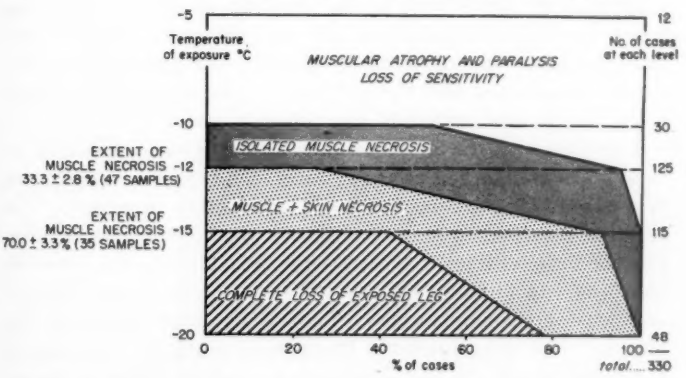
Muscle necrosis and atrophy were determined by weight. For this purpose, 4 muscles or muscle groups of both the exposed and the healthy hind leg were dissected: (1) musculus tibialis anticus plus musculus extensor digitorum longus, (2) musculus flexor digitorum longus, (3) musculi peronaei—brevis and longus, and (4) musculus triceps surae—gastrocnemius plus soleus. It was assumed that the weight of the muscles of the unexposed leg was a reasonable basis for comparison with regard to atrophy. The necrotic parts were cut away with scissors, weighted, and the amount of necrotic tissue determined in percentage of the total muscle weight of the exposed leg.

In most cases atrophy and necrosis of the muscles were simultaneously present. Necrosis caused an increase in volume and weight of the muscle tissue. Contrarily, the surviving part of the muscle showed a marked reduction in mass and volume. The calculated percentage of necrosis was, therefore, always higher than its actual value. But since the underlying function was continuous, a comparison of the values was nevertheless possible. The calculation of a correction factor was not considered necessary for our purpose.

Results

The results are reported according to the sequence of increasing injury, that is, the decrease in temperature to which the animals were exposed (see Graph 1).

GRAPH 1. RESULTS OF EXPOSURE TO DIFFERENT BATH TEMPERATURES FOR 30 MINUTES



Important observations of the sequence of increasing injury are graphically shown for temperatures of –5° to –20° C. The injury progresses in the following sequence: functional changes (loss of sensitivity and paralysis) and atrophy, muscle necrosis, skin necrosis in addition to muscle necrosis, and finally complete loss of the leg.

For the purpose of this paper, there were three characteristic groups of resulting damage, with some overlapping, of course. The first group comprised degrees of injury with predominantly functional changes, that is, the animals suffering these injuries usually displayed complete clinical recovery with regard to

the exposed limb. Under our conditions with an exposure time of 30 minutes, functional changes occurred in the temperature range from + 5° to — 5° C. The second group consisted of degrees of injury which resulted in permanent and irreparable destruction of parts of the exposed limb without superficial (skin) necrosis. This occurred in our investigation at temperatures of — 10° to — 12° C. The third group included injuries which usually resulted in superficially visible loss of smaller or larger parts of skin and muscle of the exposed limb. Bath temperatures lower than — 12° C. produced this result under our standard conditions. Only the third group corresponded to what traditionally is called "frostbite."

1. *Functional Changes*

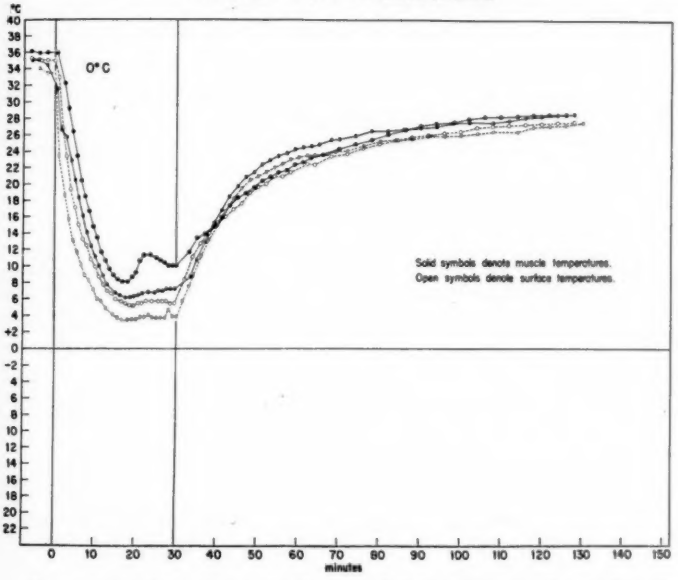
Exposure to +5° C. for 30 minutes (12 animals). The animals did not develop edema of the exposed leg. There was no visible atrophy, but at autopsy there was generally a slight reduction in the weight of the muscles of the exposed legs when compared to those of the opposite side. Immediately after exposure the muscular power in foot and leg was distinctly diminished and the reaction to pinprick dulled. These signs disappeared within 3 days after exposure.

Exposure to 0° C. for 30 minutes (12 animals). Little or no swelling was observed after exposure. The reaction to pinprick was absent in some animals and hyperactive in others. This reaction returned to normal within 1 week. A moderate atrophy became visible after 1 week, especially in the anterior muscle group (tibialis anticus and extensor digitorum longus) and was verified at autopsy. Four weeks after exposure this atrophy had disappeared. There was marked reduction of muscular power in foot and leg. In several cases a hyperextension in the ankle joint was observed, obviously caused by a more severe damage to the anterior muscles which normally flex the ankle. The spreading reflex of the toes was diminished or absent in most animals. These muscular signs largely disappeared after 4 weeks.

The actual temperatures in the muscle at the center of the leg and on the skin surface as determined by the thermocouple unit during this degree of exposure are represented in Graph 2 for 2 animals.

Exposure to —5° C. for 30 minutes (12 animals). Functional changes again predominated, but atrophy was more severe than in those animals exposed to 0° C. Minimal swelling was observed after exposure. With the disappearance of this swelling on the second day, distinct muscle atrophy became visible in some animals. The reaction to pinprick was either absent or hyperactive but returned to normal after 1 week.

GRAPH 2: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO 0°C FOR 30 MINUTES (two animals)

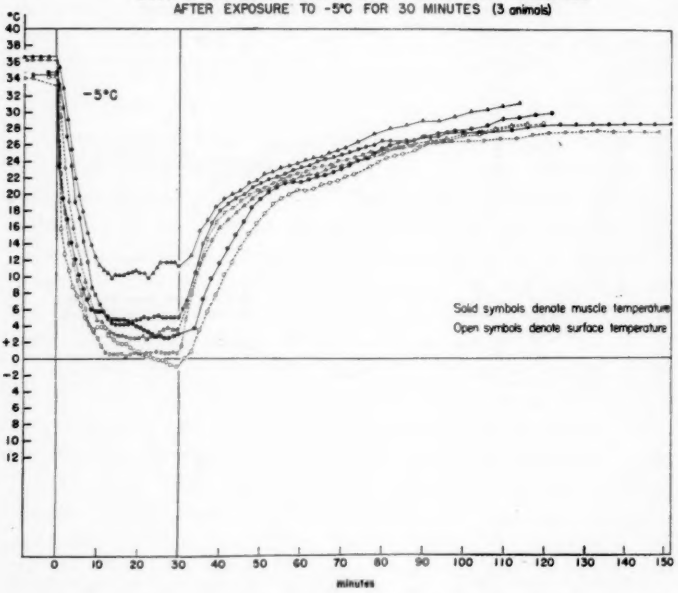


The muscular power in foot and leg was greatly reduced. The spreading reflex of the toes could not be elicited. The feet were in some cases hyperextended in the ankle by the overwhelming counteraction of the calf muscles, and in some cases slight but distinct foot drop existed temporarily. These muscular signs generally disappeared after 4 weeks. But in some cases changes in the exposed leg were observed up to 2 months after exposure, that is, decreased power, induration of the muscles, increased and inelastic resistance to passive

movement of the ankle, and abnormal attitudes of the foot due to hyperextension of the ankle joint. At autopsy, 1 week after exposure, all animals showed muscle atrophy of the exposed leg but no necrosis.

During exposure to this degree of injury the muscle temperatures in the triceps surae as well as those of the skin usually stayed above 0° C. (Graph 3).

GRAPH 3: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO -5°C FOR 30 MINUTES (3 animals)



2. *Atrophy and Isolated Necrosis of Muscle*

Exposure to —10° C. for 30 minutes (30 animals). With this degree of injury we found a further increase of the signs observed in the foregoing group. There was severe swelling after exposure. An impressive atrophy of the whole leg became visible as soon as the swelling disappeared (3 to 4 days). The reaction to pinprick was usually absent but reappeared within 2 months in most cases. There was almost complete muscular paralysis with foot drop and absence of the spreading reflex

of the toes. The atrophy and many of the functional signs of damage to the muscles existed at the end of the observation period of 2 months.

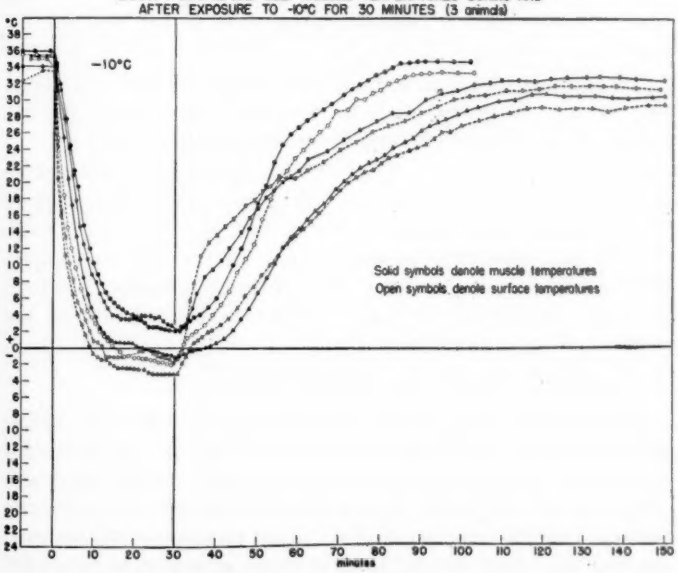
The skin also showed signs of atrophy. It was thinner, softer, and shinier than normal.

Of 16 animals autopsied after 8 days, 8 displayed gross necrosis of the musculus tibialis anticus and extensor digitorum longus without any gangrene of the overlying skin. This necrosis was usually limited to the distal half of these muscles.

The occurrence of skin necrosis in one of these 30 animals was probably accidental and, therefore, not further considered.

Exposure to — 10° C. resulted in lowest skin temperatures of about — 2° to — 3° C. The temperatures in the center of the triceps surae were usually above or around 0° C. (Graph 4). The temperatures in the superficial layers of the muscle were, of course, close to those of the skin.

GRAPH 4: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO -10°C FOR 30 MINUTES (3 animals)



Exposure to — 12° C. for 30 minutes (125 animals). The overwhelming number of animals showed the same changes

as the animals exposed to — 10° C. but to a greater degree and, in addition, other damage. In 99 autopsied animals the muscles of the leg were examined, and in 93, gross necrosis was found. In 47 of these the amount of muscle necrosis was determined quantitatively. The remaining 6 animals displayed a severe atrophy of the muscles.

The muscle necrosis involved primarily the anterior muscle group (tibialis anticus and extensor digitorum longus). The weights of these two muscles comprise approximately 25 per cent of the total muscle weight of the leg as used in these experiments. Small areas of necrosis also involved the flexor digitorum longus, the peronaei muscles, and, occasionally, the distal portion of the triceps surae to account for the average total muscle necrosis of 33.3 per cent in 47 animals.

Of 125 animals, including the 99 mentioned above, 97 did not develop superficial (skin) necrosis but suffered from practically complete muscular paralysis and loss of surface sensation. Atrophy of the skin was present as in the animals exposed to — 10° C.

The 28 remaining animals of this group suffered superficial necrosis as well as necrosis of the muscles. According to the end results, therefore, they belong in the next group.

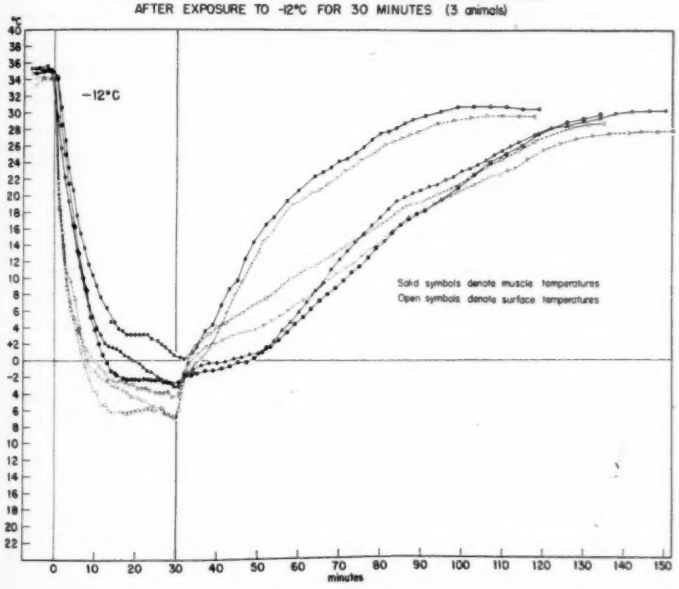
During exposure to — 12° C. the surface temperatures were lowered to — 4° to — 7° C. The deep muscle temperatures in the triceps surae remained between 0° and — 3° C. (Graph 5). At this exposure the temperature in the skin and superficial muscle layers passed — 6° C. in some animals, the temperature considered by Lake (1917) to be critical for the destruction of organic matter.

3. *Superficial (Skin) Plus Muscle Necrosis*

Exposure to — 15 C. for 30 minutes (115 animals). Most of the animals displayed the picture which is usually called "frostbite." The extent of necrosis varied. Forty-seven animals lost the exposed leg completely and 58 suffered from partial loss of varying degree. The remaining 10 animals showed the same results as described for exposure to — 10° or — 12° C., that is, muscular and sensory paralysis with gross necrosis of muscle but without superficial (skin) necrosis.

In these experiments not a single case was observed with isolated skin necrosis, that is, gangrene of the skin without muscle necrosis. The necrotic areas of the skin were almost without exception directly over necrotic muscle. The area of necrotic muscle distinctly exceeded that of the overlying skin necrosis in every direction, either at the demarcation line in cases with complete loss of the leg or at the periphery of necrotic areas in instances of limited necrosis. In 35 animals the average muscle necrosis was 70.0 ± 3.3 per cent.

GRAPH 5: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO -12°C FOR 30 MINUTES (3 animals)



The most extensive partial skin necrosis just short of losing the whole leg did not include the 2 to 3 cm. just above the ankle, which has no underlying muscle, but involved the rest of the skin to the immersion line. According to the physical conditions the area just above the ankle was most severely exposed, but escaped gangrene.

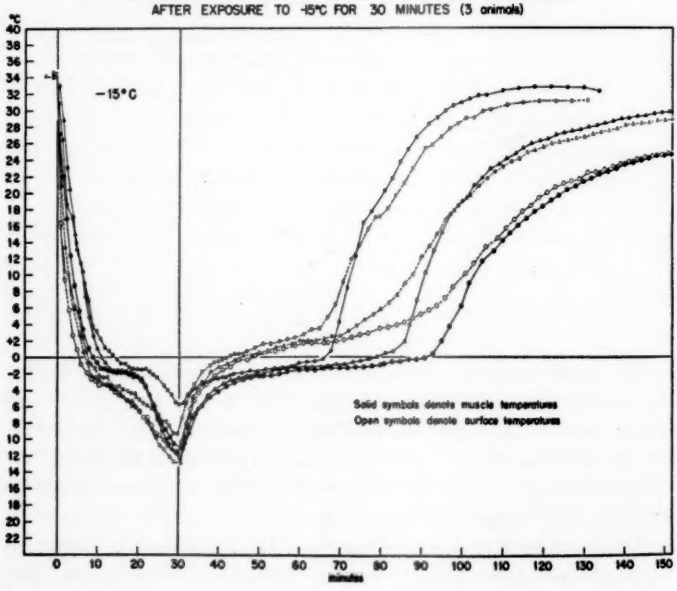
All animals that did not lose their legs exhibited a complete sensory and motor paralysis. The spreading reflex of the toes

was always lost, and in most cases did not reappear during the period of observation (2 to 3 months). In practically all animals foot drop of varying degree existed. The sensory paralysis disappeared in many cases after approximately 2 months. In others it existed 3 months after exposure.

Some cases showed partial restoration of function after approximately 2 months. Complete or severe foot drop improved, obviously by partial replacement of the anterior muscles with fibrous tissue, but with little regain of function.

The temperature curves for exposure to — 15° C. differed essentially from all preceding graphs. All curves at this level showed a delay in the temperature change at approximately — 2° C. during cooling as well as during thawing, reflecting the energy loss during freezing and thawing. The deep muscle temperatures almost reached the same level as the skin temperatures, and these were close to the temperature of the bath, namely, — 10° to — 12° C. Graph 6).

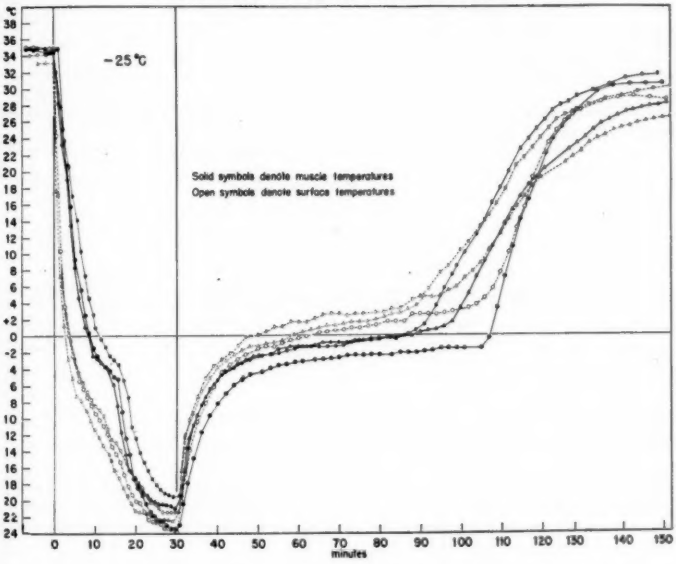
GRAPH 6: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO -15°C FOR 30 MINUTES (3 animals)



Exposure to temperatures lower than — 15° C. for 30 minutes (60 animals). This degree of injury resulted in a rapidly increasing tendency toward complete necrosis of the leg. Otherwise, there were no changes from the pattern observed after exposure to — 15° C. Of 60 animals surviving exposure to temperatures lower than — 15° C., 49 lost the leg completely and 11 suffered loss of large parts of the leg.

Exposure to — 25° C. resulted in skin and deep muscle temperatures which almost coincided with temperature of the exposure bath (Graph 7).

GRAPH 7: SURFACE AND DEEP MUSCLE TEMPERATURES DURING AND AFTER EXPOSURE TO -25°C FOR 30 MINUTES (3 animals)



Reproducibility of the standard injury. The following tables show the reproducibility of the degree of injury in groups as they have been actually used in this investigation. In our experience, groups of 12 animals are sufficient to ascertain the particular degree of injury. Table 1 shows results after exposure to — 12° C. under the headings "no superficial necrosis," "partial surface necrosis," and "complete necrosis."

Table 2 shows the same for exposure to — 15° C. As can be seen from the tables, there is no single group in which the degree of injury is not characteristically represented.

Table 1

Reproducibility of the Standard Injury

Results in Different Groups Exposed to -12°C. for 30 Minutes

Date	No.	Died	Complete Necrosis of Exposed Leg	Partial Surface Necrosis	No Superficial Necrosis	Total
Sept 1949	FR 293-304	1	-	2	9	12
Sept 1949	FR 305-316	-	-	-	12	12
Oct 1949	FR 328-340	-	-	3	9	12
Nov 1949	FR 341-352	-	-	1	11	12
Nov 1949	FR 359-370	-	-	3	9	12
Dec 1949	FR 371-382	-	-	5	7	12
Dec 1949	FR 383-394	-	-	2	10	12
Total		1	0	16	67	84
Result in per cent		1%		19%	80%	

Table 2

Reproducibility of the Standard Injury

Results in Different Groups Exposed to -15°C. for 30 Minutes

Date	No.	Died	Complete Necrosis of Exposed Leg	Partial Surface Necrosis	No Superficial Necrosis	Total
Oct 1948	FR 49-60	2	5	5	-	12
Oct 1948	FR 61-72	1	3	6	2	12
July 1949	FR 211-234	4	8	11	1	24
Aug 1949	FR 281-292	2	2	8	-	12
Dec 1949	FR 395-406	2	5	4	1	12
Jan 1950	FR 407-430	2	10	11	1	24
Total		13	33	45	5	96
Result in per cent		13%	35%	47%	5%	
For surviving animals only			94%		6%	

The extent of muscle necrosis at these two levels of injury is shown in Table 3. Especially striking are the consistency of results in the groups exposed to the same experimental conditions and the difference between the extent of final injury at the two temperature levels.

Table 3

Reproducibility of the Standard Injury

Extent of Muscle Necrosis After Exposure to -12°C. for 30 Minutes

	Average Value	Number of Animals
FR 293-304	28.5 ± 4.5%	11
FR 305-316	31.1 ± 5.1%	12
FR 329-340	38.8 ± 6.5%	12
FR 341-352	34.0 ± 5.6%	12
	33.3 ± 2.8%	47

Extent of Muscle Necrosis After Exposure to -15°C. for 30 Minutes

FR 395-406	70.4 ± 7.4%	11
FR 407-418	73.5 ± 4.3%	12
FR 419-430	66.1 ± 5.5%	12
	70.0 ± 3.3%	35

Discussion

It has been customary in experimental investigations on local cold injury to use conditions of exposure which resulted in the same degree of injury in all animals. It is obvious from the general features of Graph 1 that such a standard injury is not very useful. In order to produce injury of the same severity in all animals, we have to choose conditions which lead to complete necrosis in all animals and which are for the

overwhelming number more severe than necessary for the production of this injury. That means the uniformity of the standard injury of this type is more apparent than real. Further, this method is not sufficiently sensitive for the evaluation of therapeutic measures, since a considerable number of animals will always be damaged beyond possible recovery.

With our method of standardization we can choose levels of injury in which almost every animal stays within a range which does not preclude recovery. This procedure is, therefore, more economical. Influences of factors which alter the course in either direction, improving or worsening, become visible in a shift of the distribution of the different degrees of injury. The quantitative determination of the extent of muscle necrosis increases the sensitivity of the method considerably.

For the two levels of injury used most in our investigations, namely, from exposure to — 12° and — 15° C., the extent of muscle necrosis was determined. Exposure to — 12° C. for 30 minutes caused, on the average, 33 per cent necrosis of the leg muscles. Exposure to — 15° C. resulted in necrosis of 70 per cent of the muscles. This steep increase in the extent of muscle necrosis with a relatively small decrease in bath temperature showed that these conditions of exposure were in a critical range.

The results of these experiments indicate that exposure of an extremity for 30 minutes to cold of such severity as to produce temperatures not below freezing within the tissue causes primarily functional changes of the limb. The rather pronounced atrophy found after such exposures is an exception to the above statement.

The cause of the muscle atrophy is not known and will require further investigation. The possibilities are that it is due to (1) direct action of the cold on muscle fibers, (2) injury to the motor nerves or end plates, (3) disuse of the injured leg, and (4) ischemia resulting from vascular changes. Likewise, the cause of the muscular paralysis may be due either to nerve or end plate injury or to direct action of cold on the muscles. Conduction in mammalian nerves exposed to cold has been investigated by Denny-Brown and collaborators

(1945). They found in cats that temperatures in the sciatic nerve as high as + 5° to + 6° C. for 120 minutes resulted in temporary complete motor and sensory paralysis. When the nerve temperature was maintained at + 8.0° to 11.6 ° C. for the same period, little or no functional changes were noted. Obviously, conditions were different in our animals since the exposure time was but 30 minutes.

It is interesting that, in other experiments, the animals maintained excellent motor function of severely frostbitten legs immediately after rapid thawing in 42° C. water for 5 minutes. However, some of these animals developed paralysis the following day.

Under our experimental conditions, necrosis of skin or muscle did not develop when tissue temperatures above freezing prevailed. These conditions were present during exposure to bath temperatures of — 5° C. and above.

Exposure to bath temperatures of — 10° C. and below produced tissue necrosis. The necrosis appeared first in the muscle and second in the skin with decreasing temperatures. This is direct evidence that muscle is more sensitive to cold than skin, since for any given exposure temperature, the latter gets colder than the former. This phenomenon was well demonstrated by exposure to — 12° C. which resulted in muscle necrosis in 95 per cent of the animals and skin necrosis in only 22 per cent. This observation is strong evidence against the theory that tissue necrosis after local cold injury is secondary to vascular changes. At — 15° C. and below, injury of such severity was produced as to cause extensive necrosis of all tissues and often complete loss of the leg.

It should be mentioned that in these experiments the temperature measurements were not obtained from the areas in which necrosis first developed. It is very probable that temperatures in the anterior muscle group of the leg became lower during exposure than in the triceps surae.

It is interesting to note that some muscle and skin temperatures recorded during exposure showed evidence of heat regulation (Graphs 2, 3, 4, 5). This apparently is of the same nature as the skin temperature regulation during exposure to cold first discovered by Lewis (1930) and subsequently verified

and described by Kramer and Schulze (1948). To our knowledge this phenomenon has not been observed previously in muscle.

Summary and Conclusions

The sequence of functional and anatomical changes in rabbit legs following exposure to various degrees of cold injury is reported. Temperatures attained by the skin and deep muscle during these exposures are also given.

Three characteristic degrees of injury resulted from exposure to + 5° to — 40° C. for 30 minutes. (1) Functional changes occurred from exposure to + 5°, 0°, and — 5° C. and were temporary. The abnormalities noted were loss of muscular power in leg and foot, absence of response or hyperactive reaction to pinprick, loss of spreading reflex of the toes, and in some cases hyperextension of the ankle joint. Muscle atrophy was observed in the more severe of these exposures and represented extension into the second category of increasing injury. (2) Atrophy and isolated necrosis of muscle occurred from exposures to — 10° to — 12° C. in addition to the functional changes described above. (3) Skin necrosis plus the abnormalities noted under *(1)* and *(2)* occurred from exposure to temperatures below — 12° C. Often the end result of these injuries was complete loss of the leg.

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A CRITICAL ANALYSIS OF NEOMYCIN AS AN INTESTINAL ANTISEPTIC

EDGAR J. POTH, RICHARD G. MARTIN, STANLEY M. FROMM, ROBT. I. WISE, AND CHIN MIN HSIANG*

We recently reported on neomycin as an intestinal antiseptic. This antibiotic has the most favorable antibacterial characteristics of the known antibiotics in the bowel. The most serious defect of the antibiotics as intestinal antiseptics is the tendency to establish resistant strains of bacteria. Neomycin permits only rarely the development of resistant bacterial strains.

Neomycin[1] was first produced in a culture of Streptomyces (No. 3535), which is closely related to Streptomyces Fradaie, by Waksman and Lechevalier.

Neomycin is a water soluble polybasic acid with an equivalent weight of approximately 150, forms crystalline salts with parahydroxyazobenzene-para-1-sulfonic acid and ammonium reineckate and sulphate which is a water soluble, amorphous powder having a specific rotation of +55. The sulphate has a potency of 200 Waksman units per milligram. Neomycin, unlike most other antibiotics, is stable in alkaline solution. Chromatographic studies show this fraction of neomycin to be a single compound. The aqueous solution is stable at 37° C. for a minimum of one month. It possesses a high degree of antibacterial activity against a wide variety of gram positive and gram negative bacteria (Waksman, et al.; Poth, Fromm, Wise and Hsiang).

Preliminary observations made by Dr. Reagan Howard Gibbs of the Pediatrics Department of this institution would indicate that neomycin has no antibacterial activity against the shigella organisms.

Procedure

The optimum adult dosage of neomycin as previously reported by Poth, Fromm, Wise and Hsiang is one gram every

*From the Surgical Research Laboratory, Department of Surgery, The University of Texas Medical Branch, Galveston, Texas. Received for publication May 10, 1951.

[1]The neomycin used in these studies was supplied by Dr. Earl Burbidge through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

four hours. Since this compound is not toxic when administered orally, further studies were done on man directly. Blood, urine and fecal specimens were collected to determine absorption and excretion. During the early phases of this study, all stool specimens were studied bacteriologically and the rate at which the various bacteria were eliminated determined.

Ultimately clinical application of these findings was inaugurated. Patients requiring surgical procedures on the gastrointestinal tract were prepared for operation and the optimum conditions established.

The Antibacterial Action of Neomycin in the Bowel

The antibacterial action of neomycin in the gastrointestinal tract was studied directly on man since the oral administration of this antibiotic had been shown not to be toxic. Convalescent patients with normal gastrointestinal tracts were selected for initial studies.

Varying dosages of drug were administered at four hour intervals. When ½ gram of neomycin was administered every 4 hours, it was found that while the B. coli were promptly reduced, the growth of the Aerobacter aerogenes was not inhibited but rather grew out in even larger numbers. If 0.75 gram of neomycin was administered on a 4 hour schedule, all organisms were usually eliminated but not invariably so.

The dosage schedule finally adopted as most satisfactory is 1.0 gram every 4 hours. This schedule was used on 30 patients with entire success without evidence of the development of resistance. Then 3 cases occurred in rapid succession of the persistant growth of Aerobacter aerogenes. These organisms proved to be highly resistant to neomycin. In addition, the administration of sulfathalidine in doses of 3.0 grams q 4 hours failed to eliminate the residual organisms from the stools of these patients. These observations indicate that, on occasion, Aerobacter aerogenes will prove resistant to neomycin. Since resistant strains of Aerobacter aerogenes have never been observed to develop following the administration of sulfasuxidine or sulfathalidine, and since neomycin administration tends to produce a degree of diarrhea, neomycin therapy was supplemented by the addition of 1.5 grams of sulfathalidine on a four hour

schedule to be administered simultaneously with the neomycin. This combined therapeutic schedule has proved successful on 154 subsequent patients. There has not been a single instance of failure to reduce the entire bacterial flora to extremely low numbers of less than 100 in 24 to 48 hours. Three days preoperative preparation has proved satifactory in the absence of obstruction. Such a short period of preparation requires that the bowel be evacuated completely and rapidly, and so castor oil is given initially.

In the absence of obstruction or other contraindications, the following schedule had been adapted:

1. Low residue diet.
2. A single dose of 30 to 60 cc of castor oil is given. 1.0 gram of neomycin sulphate together with 1.5 grams of sulfathalidine are given orally on a 4-hour schedule. First dose of the antibiotic and sulfonamide is given at the same time as the purgative.
3. Routinely the stools are cultured at the end of each 24 hour period.
4. Preoperative preparation is for 3 days.
5. Usually within 48 hours postoperatively, 1.5 grams of sulfathalidine is given on a 4-hour schedule if there is reason for postoperative medication such as the performance of a primary anastomosis of bowel.

Shortly after the bacterial flora has been reduced to extremely low numbers or to zero, the outgrowth of yeasts occur and may be mistaken for an increased growth of gram positive bacteria. Since the intestinal flora is eradicated so rapidly by this combined therapy, it might be possible to reduce the dose of the individual agents.

The indications for the use of a purge of castor oil are evident from the observation following the administration of a 1.0 gram dose of neomycin simultaneously with 5 grams of medicinal charcoal to mark the stool, Chart 1. No charcoal is present until the passage of the second stool 34 hours after administration of neomycin. Without purgation, nearly 36 hours are virtually lost in the preoperative preparation which can be saved by an initial cathartic.

It will be observed that there is a significant drop in the bacterial count before charcoal marked feces is passed. Apparently the soluble drug is carried along the bowel in the liquid portion ahead of the charcoal marked solids, because parenteral administration of neomycin has no antibacterial action in the bowel. Also, it is required that the drug be present in sufficiently large quantity to act bactericidally, since the flora in the feces ahead of the charcoal has been reduced significantly.

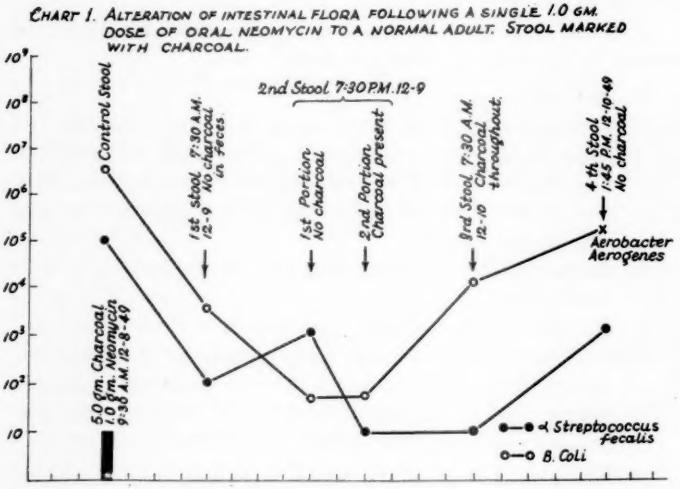

Control Stool
1st stool 7:30 A.M. 12-9 No charcoal in feces.
2nd Stool 7:30 P.M. 12-9
1st Portion No charcoal
2nd Portion Charcoal present
3rd Stool 7:30 A.M. 12-10 Charcoal throughout.
4th Stool 1:45 P.M. 12-10-49 No charcoal
Aerobacter Aerogenes
5.0 gm. Charcoal 1.0 gm. Neomycin 9:30 A.M. 12-8-49
Streptococcus fecalis
B. Coli

CHART 1. ALTERATION OF INTESTINAL FLORA FOLLOWING A SINGLE 1.0 GM. DOSE OF ORAL NEOMYCIN TO A NORMAL ADULT. STOOL MARKED WITH CHARCOAL.

Chart 2 shows in detail what occurs in man when 4.0 grams of neomycin are given orally in 1.0 gram doses at 4-hour intervals. Besides illustrating the relative sensitivity of the various bacteria, this chart indicates that neomycin exerts a profound effect on the bacterial flora of the bowel for 24 to 36 hours after administration of the last dose of the drug.

Absorption and Excretion

The fate and distribution of ingested neomycin was investigated by determining the concentration of antibiotic in the blood, the quantity excreted in the urine and the amount recoverable from the feces. These obeservations were made after the subject had received 1.0 gram of neomycin by mouth each four hours for three days.

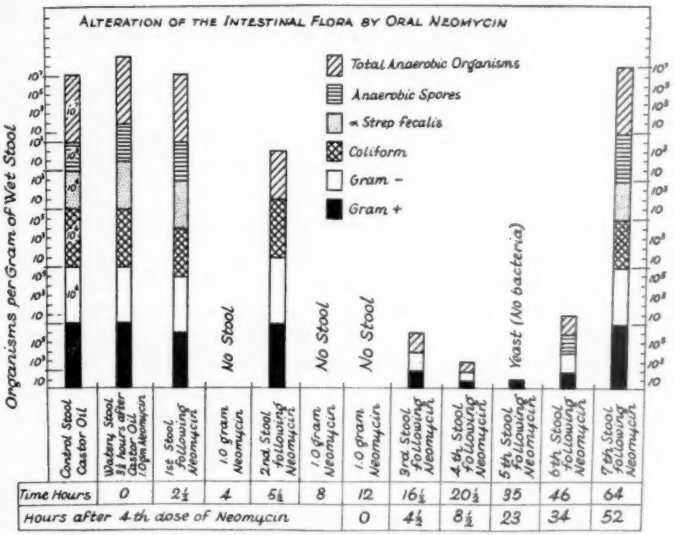

Alteration of the Intestinal Flora by Oral Neomycin
Total Anaerobic Organisms
Anaerobic Spores
α Strep Fecalis
Coliform
Gram −
Gram +
Organisms per Gram of Wet Stool
No Stool
No Stool
No Stool
Yeast (No bacteria)
Control Stool Castor Oil
Watery Stool 11 hours after Castor Oil 10 gm Neomycin
1st Stool Following Neomycin
1.0 gram Neomycin
2nd Stool Following Neomycin
1.0 gram Neomycin
1.0 gram Neomycin
3rd Stool Following Neomycin
4th Stool Following Neomycin
5th Stool Following Neomycin
6th Stool Following Neomycin
7th Stool Following Neomycin
Time Hours 0 2½ 4 5½ 8 12 16½ 20½ 35 46 64
Hours after 4th dose of Neomycin 0 4½ 8½ 23 34 52

CHART 2. Illustration of the alteration of the bacterial flora of the feces effected by the administration of 1.0 gram of neomycin orally to an adult q 4 hr. for 4 doses. The bacteria disappear in the following order—1) Streptococcus fecalis, 2) anaerobic spores, 3) coliform and 4) gram negative, gram positive and all anaerobic organisms. Yeasts grow out when the bacteria are eliminated.

Blood specimens were taken 2 hours after a dose of drug or midway between two doses. The assay method is a modification of the procedure of Loo, Skell and Thornberry. Five ml of agar seeded with spores of B. subtilis were poured into a flat bottom petri dish and allowed to cool. Undiluted blood serum and serum diluted with an equal volume of 0.5 molar phosphate buffer (pH 7.85) were prepared. Filter paper discs ¼ inch in diameter were saturated in the prepared sera and transferred directly to the agar plates. After storing in the refrigerator for 12 hours to allow diffusion of the neomycin into the agar, the plates were incubated at 30° C. for 18 hours and the diameter of the zones of inhibition measured and compared against zones obtained with known concentrations of neomycin in 0.5 molar phosphate buffer (pH 7.85). The known dilutions contained 32, 16, 8, 4 and 1 and 0.5 micrograms of neomycin per ml.

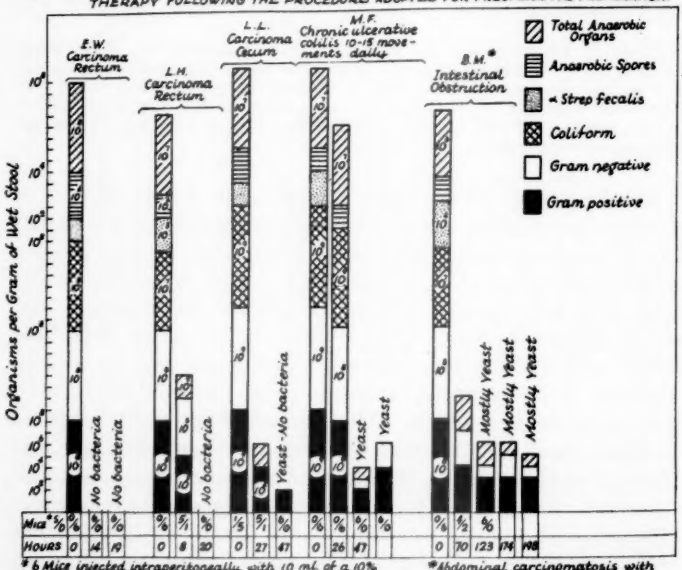
The concentration of neomycin in blood sera varied from a trace to 80 micrograms per mil. Obviously these concentrations are very low.

Twenty-four hour urine specimens were collected and an aliquot part assayed for neomycin. Dilutions of 1 to 5, 1 to 10 and 1 to 20 with 0.5 molar phosphate buffer (pH 7.85) usually gave satisfactory preparations for assay by the method described for blood serum.

An average of 3% of the ingested antibiotic could be recovered from the urine.

The concentration of neomycin in feces was determined on 24 hour collections. A 1 to 100 suspension was first made, followed by further dilutions of 1 to 200, 1 to 400, 1 to 800 and 1 in 1600 in 0.5 molar phosphate buffer and tested by the assay method outlined for blood serum. The dilution factor is so large that the final result is quite inaccurate, but the rough estimation would indicate that the major portion of the antibiotic is passed in the feces unaltered.

CHART 3. ALTERATION OF THE INTESTINAL FLORA BY THE COMBINED NEOMYCIN-SULFATHALIDINE THERAPY FOLLOWING THE PROCEDURE ADOPTED FOR PREOPERATIVE PREPARATION.



* 6 Mice injected intraperitoneally with 10 ml. of a 10% suspension of each fecal specimen obtained from patients at the times indicated. ⅚ = Survival/Death.

*Abdominal carcinomatosis with nearly complete obstruction of both small and large bowel.

Mouse Test for Alteration of Feces

An in vivo test for the effectiveness of an intestinal antiseptic was devised as follows: One ml of a 10% suspension of human feces is injected into the peritoneal cavity of a 20 gram mouse. This quantity is equivalent to the the introduction of 300 grams of feces or 3000 ml of a 10% suspension of feces into the peritoneal cavity of a 60 kg man.

Such a procedure utilizing ordinary human feces results in the death of 100% of the mice in from 6 to 24 hours.

Immediately after elimination of the bacteria from human feces by the oral administration of neomycin, 100% of the injected mice survive indefinitely without untoward effects, Chart 3.

The Bactericidal and In Vitro Activity of Neomycin

The addition of varying amounts of neomycin to 10% suspensions of human feces gave the following results:

50 micrograms of neomycin per ml.
6 mice injected
5 died within 20 hours
1 died in 27 hours

500 micrograms of neomycin per ml.
6 mice injected
4 died within 24 hours
1 died in 29 hours
1 died in 36 hours

5,000 micrograms of neomycin per ml.
6 mice injected
1 died in 36 hours
5 survived more than 48 hours

50,000 micrograms of neomycin per ml.
6 mice injected
6 died within 1 hour

(LD 50 of neomycin for 20 gram mouse equals 7,000 micrograms)

The academic question might be raised that the mice injected with suspensions of feces from treated individuals lived only

because of the neomycin present and not due to alteration of the bacterial flora. The highly practical answer is that the mice injected with the altered feces survived, and it might be inferred that contamination of the peritoneal cavity of man with large quantities of such feces will result in survival regardless of the reason for such survival. In fact we have observed two cases where gross continued contamination of the peritoneal cavity occurred. Both patients remained afebrile and peritonitis did not develop.

The Use of Neomycin in Emergency Abdominal Surgery

The demonstration of the rapid bactericidal action of neomycin to sterilize feces and its effectiveness in protecting animals after the intraperitoneal inoculation with rapidly lethal quantities of fecal suspension suggests immediately the possibility that this highly soluble, sparingly absorbed antibiotic may be useful in urgent abdominal surgery of intestinal obstruction, stab wounds, gunshot wounds, or other intra-abdominal catastrophies involving the gastrointeric tract.

Mongrel dogs were subjected to laparotomy without any preceding treatment designed to empty the bowel or modify its bacterial flora. The entire small and large bowel was filled with 1% aqueous neomycin solution and subsequently specimens were aspirated and cultured for bacteria. Ususally within an hour the bowel contents were found to be sterile.

The transverse colon was then divided and resutured by the open technique. Healing proceeded without complications and simulated primary tissue repair.

This procedure was then applied to patients. Five cases have been treated as follows:

Gunshot wound of abdomen with large perforations in transverse colon, ileum and jejunum and gross fecal soiling: *1 case.*

Mesenteric thrombosis involving ileum and requiring resection of a three foot length of bowel: *1 case.*

Three cases of complete obstruction of colon.

Carcinoma of hepatic flexure,
Carcinoma of transverse colon, and
Carcinoma of sigmoid

The distended loops of bowel in two of the obstructed cases were evacuated by opening the bowel and emptying the contents into a sterile wash basin. After occluding the opening with a clamp, the entire bowel was filled with 1% aqueous neomycin solution both proximal and distal to the obstruction. Specimens taken from the bowel after 30 minutes and transferred to culture media immediately failed to grow bacteria. In a third case of obstruction, in which the bowel contents were fluid, about half the contents were aspirated and the liquid replaced with an equal quantity of 2% neomycin solution at various levels of the small and large bowel through a 14 gauge needle.

In the instance of the gunshot wound of the abdomen, the large perforations were isolated between clamps, the lumen filled with 1% neomycin solution and the surface of the segment sponged with the same 1% solution. The entire small and large bowel was filled with 1,000 cc of 1% neomycin solution. The general abdominal cavity irrigated with normal salt solution, and the perforations closed. The defect in the transverse colon required resection of a small segment and primary end-to-end anastomosis. Postoperatively the maximum temperature elevation in no case exceeded 1° C. The postoperative course was smooth and uncomplicated in all instances. Although primary resection and anastomosis was performed in all three cases of carcinoma with obstruction, it is not advocated that primary bowel suture be done when the bowel is edematous.

These early trials would indicate the usefulness of neomycin in urgent gastrointestinal surgery in various selected circumstances where the bowel cannot be prepared preoperatively. This regimen should be applicable in the handling of many cases of obstruction and acute traumatic lesions of the bowel. Neomycin should not be placed in the peritoneal cavity in quantities exceeding 0.5 gram because of its toxicity when given parenterally.

Toxicity

Both dogs and man have been maintained on a daily oral dosage schedule of 0.1 gram of neomycin per kg. of body weight for six weeks without any evidence of kidney damage as reflected by urine examination. No other untoward effects were

observed. Dogs were sacrificed and the tissues studied both grossly and microscopically without finding any abnormalities.

Specimens of bowel removed at operation have shown no irritation of the mucosa. Nausea does not accompany the oral administration of this antibiotic.

There is an increase in the number of bowel movements, and these passages are thin, greenish in color and odorless. The volume of stool is not appreciably increased. There is some increased mucus in the stools. There is no appreciable increase in peristaltic activity. There is no demonstrable alteration of the bleeding time, clotting time or prothrombin time even after prolonged administration of the drug with sustained absence of bacteria in the bowel. Yeasts are always present. Increased bleeding has not been observed either preoperatively or postoperatively.

Bowel Healing as Influenced by Neomycin

Previous studies (Poth, et al.) have demonstrated that the character and rate of healing of the colon is strikingly modified following alteration of the bacterial flora in the bowel. The healing at the site of anastomosis of the colon closely simulates primary tissue repair when the bacterial flora has been eliminated. These characteristics are most prominent on the third postoperative day when studied on the dog.

Treatment of mongrel dogs fed on a horsemeat diet containing neomycin in such quantity that each animal received on a 4-hour feeding schedule, 0.2 gram per kilo of body weight daily and resulted in practically complete elimination of bacteria from the gastrointestinal tract within 3 days. Control animals received the horsemeat diet but no drug except magnesium sulphate to evacuate the bowel. After 3 days preoperative treatment, laparotomy was performed, the midcolon transected and then anastomosed by introducing a single, continuous, through and through inverting suture of 00 chromic catgut. An open technique was used without any precautions taken to avoid spillage of feces or gross contamination of the peritoneal cavity.

On the third postoperative day the animals were sacrificed. The anastomosis was recovered, examined grossly, the mucosal surface photographed in color and tissue taken across the suture line for microscopic study. Grossly the control specimens show

edema of the mucosa 3 to 5 cm distant from the anastomosis. The cut edges of the bowel are necrotic. Extensive edema, massive polymorphonuclear infiltration and tissue necrosis are evident on microscopic examination. Fibroplasia is absent and ingrowth of vascular capillaries is suppressed.

Following elmination of the intestinal bacteria, edema is minimal and does not extend more than one cm from the line of anastomosis. The cut edges of the bowel are clean; not grossly necrotic. The process of repair is that of primary healing. Microscopic examination shows extensive fibroplasia and ingrowth of vascular capillaries. Polymorphonuclear infiltration and edema occur to only a moderate degree in close proximity to the line of suture.

The importance of studying the effect of new antibiotics and chemotherapeutic agents upon the healing process prior to their use in human treatment of preoperative bowel preparation cannot be overemphasized. It is entirely possible that any one of these agents might suppress healing and so expose the patient to added hazards.

It is clearly evident from the experimental observations made upon the effect of altering the bacterial flora of the intestinal tract, that healing of the suture line in the colon of the dog is favored and accelerated by the oral administration of neomycin.

Effect of Oral Neomycin Administration on the Clotting Mechanism

It should be expected that the profound retardation of the growth of bacteria in the bowel by the oral administration of neomycin or the combined neomycin-sulfathalidine therapy would alter the clotting mechanism of the blood. Careful studies on both patient receiving neomycin alone and the combination of neomycin and sulfathalidine, even for periods as long as six weeks, have failed to show evidence that the bleeding, clotting and prothrombin times have been prolonged in the slightest. The yeasts which grow out in increasing numbers as the bacteria are eliminated may synthesize vitamin K in sufficient quantities to offset any deficit imposed by the elmination of the bacteria.

It is not felt necessary to give vitamin K except in those instances where the patient has a prolonged prothrombin time, bleeding tendency, jaundice or decreased plasma proteins before

neomycin-sulfathalidine therapy is begun or when the patient is receiving either no food by mouth or only highly purified diet such as split proteins. This subject has been studied extensively and will be reported upon elsewhere.

Discussion

The tendency for development of resistant strains of bacteria is not pronounced in the case of neomycin, but such development has been observed in three instances with the persistence of Aerobacter aerogenes, which furthermore did not respond to subsequent sulfathalidine and sulfasuxidine therapy. It is, therefore, desirable to add a supplementary agent which will prevent the establishment of resistant bacterial strains, Chart 3.

Sulfathalidine was chosen because the development of resistant strains of Aerobacter aerogenes has never been observed with this drug. Furthermore, neomycin tends to produce thin watery stools and sulfathalidine is more active than sulfasuxidine under these conditions. Also, sulfathalidine tends towards formation of a ropy tenacious stool and will partially counteract the thin watery stools resulting from neomycin administration.

This combined therapy permits adequate preparation of the uncomplicated case for colon surgery in 3 days as compared to 7 days for sulfathalidine when used alone. We are inclined to view the preparation using the combined therapy as being due to the action of neomycin while sulfathalidine acts to prevent the development of a resistant strain especially Aerobacter-aerogenes. It is true that the drop in the bacterial population of the bowel is somewhat more abrupt with the combined therapy than when neomycin is administered alone. The doses used may be greater than the minimum requirements, Chart 3. In fact it appears that half the dose of neomycin given with the full amount of sulfathalidine or phthaylsulfacetimide is equally as effective.

The final result of the combined administration is the rapid elimination of bacteria from the bowel. It is necessary to evacuate the bowel at the onset of therapy if rapid preparation is to result, because, without purgation, the bowels may not move for the first 24 to 48 hours so that this length of time would need

be added to the 3 days preoperative preparation period and represent lost time.

Toxicity has not been observed, but it is known that a small percentage of patients are sensitive to sulfathalidine and these patients will need be treated with neomycin alone. No definite toxicity to oral neomycin administration has been encountered.

The all important requirement that an intestinal antiseptic not retard healing is met by neomycin alone or when combined with sulfathalidine. When the intestinal bacteria have been eliminated by adequate preparation, the healing of the wound resulting at the site of bowel suture is rapid and simulates primary tissue repair.

These studies combined with the demonstration that mice will survive following the intraperitoneal injection of a quantity of altered feces equivalent to 300 grams for a 60 kilo man, demonstrate quite conclusively that, under the conditions prevailing after adequate preparation, it is not necessary to suture bowel by the the closed technique. We urge open suture of bowel because it is a simpler procedure, allows more accurate anatomical reconstruction, reduces the amount of bowel wall inverted and favors preservation of the all-important blood supply to the healing tissues. Gross soiling, contamination and subsequent peritonitis cease to be serious hazards. An illustration of the innocuousness of properly altered feces is given by a patient whom one of us saw in consultation. The patient had a far advanced carcinoma of the bladder. The patient was prepared with oral neomycin before cystectomy and ureterosigmoidostomy was performed by another surgeon. In the course of the operation, a large hole was made in the rectum accidentally and closed with one layer of continuous catgut. A penrose drain was placed through stab wounds in each flank. On the fourth postoperative day, feces and urine drained from both stab wounds and the midline incision. No feces or urine was being passed per rectum. This drainage continued for three weeks. The patient remained afebrile, the drainage was odorless and there was only slight tissue reaction around the drainage sites.

Again, we are confronted with war wounds of the abdomen, and neomycin should be of considerable value in handling these types of catastrophies. Neomycin is particularly useful because

of its rapid bactericidal activity, its broad bacterial spectrum and its limited absorption from the bowel.

Not sufficient experience has been accumulated to assess the value of neomycin alone or in combination with other antibacterial agents in the treatment of ulcerative colitis. It is a valuable agent for the preoperative preparation of patients having chronic ulcerative colitis.

Summary

Neomycin is proposed as an efficient antibacterial agent acting locally in the gastrointestinal tract following oral administration.

Data supporting the value of combined neomycin-sulfathalidine therapy is presented, and this combined therapy is advocated for the preoperative preparation of patients being subjected to colon surgery. Neomycin appears to be useful in the acute abdominal catastrophies.

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MODIFICATION OF THE ACUTE RADIATION SYNDROME:

I. Alteration by Neomycin-Sulfathalidine Therapy*

Edgar J. Poth, Fred J. Wolma, Richard G. Martin, Jack L. Smith, and Chin Min Hsiang

Total body irradiation, in sufficient doses, produces a distinct group of signs and symptoms in both animal and man and has been designated at the "acute radiation syndrome." A brief description of this syndrome is given.

There are three unique features of radiation injury: 1) The third dimensional quality or penetration of radiation injury, 2) the variation in susceptibility of different tissues to damage and 3) delayed tissue death. Also there are several factors determining the severity of the injury, including the type of radiation and its intensity and the variation in individual sensitivity to ionizing radiation.

The clinical response of patients with the acute radiation syndrome may be divided into 4 phases which occur chronologically in the course of the illness.

Phase I—The Initial Reaction: Within an hour or so after exposure to a lethal dose of ionizing radiation, the patient becomes nauseated and begins to vomit. This leads to generalized prostration which is the outstanding characteristic of this phase. Diarrhea and fall in blood pressure may occur during this phase.

Phase II—The Period of Relative Well-Being: This phase, an asymptomatic period, characteristically follows Phase I, and may last from several days to several weeks. The length of the period of well-being depends on the relative amount of radiation received.

Phase III—The Toxic Period: This period is typified by the development of *fever* (toxemia) associated with *diarrhea*, *infection*, *bleeding* and/or *loss of hair*. These symptoms develop gradually 1–4 weeks after exposure. The highest mortality rate occurs during the first two weeks after exposure. It is during

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this interval that any focus of infection, i.e. gastrointestinal tract, skin, nasopharynx, pulmonary, urinary, etc., may give rise to a fatal septicemia since the body's natural barriers to infection are minimal as evidenced by severe leukopenia.

Phase IV—Death or Prolonged Convalescence: Patients who have received lethal doses of radiation become more toxic and death ensues. Those who recover from the acute phase enter into a period of prolonged convalescence which may last for months before the patient looks and feels entirely well.

Total body irradiation causes severe damage to the reticulo-endothelial system particularly to lymph nodes, spleen, and bone marrow. The reticulo-endothelial system undergoes extensive involution. The changes detectable by laboratory methods are those seen in the peripheral circulating blood. After irradiation exposure, there may be a transient leucocytosis. However, the circulating lmyphocytes practically disappear from the peripheral blood. Following this transient polymorphonucleosis, there is a sharp decline in the leukocyte count to dangerously low levels. Because of the damage to the bone marrow, the leukocyte count remains low for several weeks followed by a very slow recovery. The red blood cells and the platelets are last to be effected and the circulating blood shows a drop in these elements after exposure. Subsequent total recovery depends upon the regeneration of the bone marrow.

Desjardins (1931) demonstrated the effects of irradiation on the gastrointestinal tract of animals and summarized the effects as follows: "Increase in tonus or spastic contraction of the intestine; mecoid degeneration, vacuolization, and desquamation of the intestinal epithelium, beginning first in the cells overlying the lymph follicles and those lining the crypts, and extending later to the epithelial investment of the villi; atrophy and disappearance of the villi; hyperemia, edema and sometimes ulceration of the mucosa; accumulation of bacteria on the denuded surface of the mucosa or in the stroma itself; leukocytic infiltration; regeneration of the mucosa in the injured portion of the bowel or proliferation of connective tissue to replace collections of cells injured beyond possible regeneration." The accumulation of bacteria on the denuded mucosal surface of the bowel and the bacterial invasion of the underlying stroma has been related on the one hand, to the thrombocytopenia which

sometimes follows irradiation and, on the other hand, to the presence of bacteria in the blood stream of irradiated animals after death.

Englestadt, in 1938, showed that a dose of 1500r or more applied over the area of the stomach caused ulcerations in the stomach of rabbits. Ulcerations occurred from 7 days to 4 weeks after irradiation and were followed regularly with such complications as hemorrhage and perforation.

Friedman and Warren (1942), producing intestinal radiation in rabbits, concluded that irradiation of the intestine resulted in an altered and damaged mucous membrane which was especially susceptible to ulceration.

Howland, Furth and Coulter, in 1950, demonstrated that the diarrheal state produced in rats and dogs by 250 KVP x-radiation could be alleviated by aureomycin or other combined antibiotics with survival prolonged 5 to 7 days. Bacteriological studies showed suppression of normally occurring gram negative organisms in the intestinal tract. Dogs treated with clinical doses of aureomycin or combined aureomycin-streptomycin showed no evidence of radiation sickness. Death in the control animals occurred between the 10th and 14th days while death in the treated animals occurred after 22nd day. All treated animals which died showed sepsis with aureomycin or streptomycin resistant organisms 48 hours prior to death.

Hammond and Miller (1950) reporting on the effect of terramycin on post-irradiation in mice, protected irradiated mice against the septicemia occurring the second week post-irradiation by the administration of terramycin unless it was caused by terramycin-insensitive bacteria. The most common terramycin-insensitive organisms encountered was Pseudomonas. The septicemia in all deaths was caused by organisms normally present in the lower bowel of stock mice.

It is conclusive that ionizing radiation, in large enough doses, will cause definite changes in the intestinal tract, namely, ulceration and hemorrhage, and that animals dying from radiation during the acute phase develop diarrhea, melena, and prostration which is followed by sepsis and death. The offending organisms have been shown to be those found in the normal flora of the gastrointestinal tract. Furthermore, it has been

demonstrated that certain antibiotics or combinations of antibiotics which alter the intestinal flora of gastrointestinal tract will prolong life until a resistant organism causes septicemia and death.

Because neomycin (Poth, Fromm, Wise and Hsiang, 1950) has been shown as an intestinal antiseptic to have a broad bacterial spectrum, rapidity of action, poor absorption from the gastrointestinal tract and no toxicity, it was selected for study as a possibly valuable agent for the treatment of the "acute radiation syndrome." Since neomycin failed to inhibit completely the growth of Aerobacter-aerogenes in all instances, it was combined with sulfathalidine and the combined therapy seemed successful in preventing the development of a resistant strain of Aerobacter-aerogenes in man (Poth, Fromm, Martin and Hsiang).

Experimental Studies

It was found that 200r total body irradiation was not fatal to dogs. LD 75 in 10 days was established by exposing dogs to 400r body irradiation half to each side of the animal. This radiation delivered as a single dose in 25 minutes from a 200 KV therapy machine was chosen as the amount to be used in all experiments reported herein.

The control animals received no medication other than routine antihelmintics. A gastric tube was passed every 4 hours to produce the same trauma to oropharynx, esophagus, and stomach as would the intubation procedures required to medicate the treated animals. The treated animals received neomycin* 100 mgm (potency—200 Waksman units per milligram) and sulfathalidine 600 mgm per kilo body weight daily, divided into 6 doses given at 4 hour intervals. Medications were given through gastric tube which was passed at time of each medication. No supportive treatment, i.e. fluids, protein, hydrolysates, whole blood, or systemic antibiotics were given to either group. The animals were weighed daily, rectal temperatures were taken twice daily (A.M. and P.M.), stools and blood were cultured daily, daily blood counts were taken, and all symptoms, i.e. anorexia, vomiting, diarrhea, melena were recorded. A

*The neomycin used in these studies was supplied by Dr. Earl L. Burbidge, through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

complete autopsy, including the central nervous system, was carried out on those animals which died. Special bacterial strains were made on tissues when indicated.

Experimental Results

Control Animals.—Four mongrel dogs, Nos. 5, 6, 7, and 8, weighing between 6.5 kg. and 8 kg. were exposed to 400r total body irradiation half to each side of the body in a single period of 25 minutes. No medications were given. All four animals developed symptoms and signs of the "acute radiation syndrome" within 72 hours. After exposure, symptoms and signs included anorexia, nausea, vomiting, diarrhea, melena, fever.

75% (3) died within 10 days post irradiation.

25% (1) survived and is still alive 2 months post irradiation. This animal so far shows only marked alopecia.

Of the 3 animals dying within 10 days, 2 showed positive blood cultures with organisms normally present in the stools.

Animal #5—Escherichia coli, streptococcus and hemolytic staphylococcus albus.

Animal #7—B-hemolytic streptococcus, and

Animal #6—negative blood culture the day prior to death. Autopsy of the 3 animals revealed ulceration and hemorrhage of the gastrointestinal tract. There were no other specific changes in the organs, i.e. adrenals, thyroid, pituitary, ovaries, testes that could be attributed to radiation. The reticulo-endothelial systems, i.e., lymph nodes, spleen showed extensive involution. The animals dying with septicemia showed bacterial invasion of the wall of the intestinal tract at the site of ulcerations.

Treated Group.—Four mongrel dogs weighing between 6.5 kg. and 8 kg. were exposed as were the controls to 400r total body irradiation half on each side of the body. These four animals received 100 mgm neomycin and 600 mgm sulfathalidine per kilo daily divided into 6 doses given at 4 hour intervals. Medications were given by stomach tube and were not started until after exposure to the radiation. These four animals received no supportive treatment other than the above medication.

There was striking suppression of organisms in the stools. All bacteria being eliminated except Aerobacter-aerogenes and

a few organisms which grew anaerobically. The changes in the blood are the same as those noted in the control animals.

All 4 of the treated animals were alive and well at the end of 10 days. The animals had good appetite, no vomiting, no diarrhea, no melena at the end of this time in comparison to 75% mortality in the control group.

One animal died on the fourteenth day.

Aerobacter-aerogenes was cultured from the blood prior to death. It is interesting to note that all bacteria were cleared from the intestinal tract with the exception of a resistant Aerobacter-aerogenes. There was slight ulceration of the stomach and rectum with extensive invasion of the wall of the bowel. This change appeared to be due to trauma caused by passage of stomach and rectal tubes.

A second animal died on the 18th day after irradiation and at the time of death the blood cultures were negative. Autopsy revealed death due to *pulmonary hemorrhage.* The gastrointestinal tract showed no lesions, no organs showed evidence of bacterial invasion of any of the body tissues.

The third animal (#12) of this group died on the 25th day post-irradiation and up to the time of death bacteremia could not be demonstrated by repeated blood cultures. This animal had no anorexia, diarrhea, or melena. Autopsy revealed no ulceration, necrosis, or bacterial invasion of the gastrointestinal tract wall. Death was due to pneumonitis and pyelonephritis.

The fourth animal remains alive and well after irradiation. The treated group of animals did not develop the typical "acute radiation syndrome," which was fully developed by the control animals. Life was prolonged by the combined neomycin-sulfathalidine therapy. This therapy did not succeed in removing all the bacteria from the gastrointestinal tract. It is practicall impossible to remove all bacteria from the bowel of the dog. One animal developed an Aerobacter-aerogenes septicemia and died 14 days after radiation. The organism was found to be in the stools and resistant to neomycin-sulfathalidine. Autopsy revealed that 2 animals dying 18 days and 25 days respectively after irradiation did not present the findings of intestinal tract ulceration, hemorrhage, necrosis, and bacterial invasion which led to fatal septicemia in the control animals. Death was due to pulmonary complications.

Conclusions

1) Exposure to total body radiation in sufficient doses, causes anorexia, vomiting, diarrhea, melena, sepsis and death. There are definite changes in the intestinal tract, i.e. ulceration, necrosis, hemorrhage, and bacterial invasion of the intestinal wall. The symptoms and signs of the acute radiation syndrome can be related to the changes in the intestinal tract.

2) The combination of high fever, toxemia and death is due to a septicemia caused by organisms normally present in the bacterial flora of the intestine.

3) The intestinal tract with its high bacterial count and pathologic changes due to radiation serves as an excellent and continuous portal of entry for pathogenic organisms into the blood stream causing fatal bacteremia.

4) Combined neomycin-sulfathalidine therapy excludes the intestinal tract as a portal of entry in the majority of cases.

5) Supportive treatment by the addition of parenteral antibacterial agents, whole blood transfusions to overcome anemia, and protein therapy are being included in the further treatment of the acute radiation syndrome now underway.

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ACCURACY OF DEATH CERTIFICATES FOR ESTABLISHING THE FREQUENCY OF CANCER AS SHOWN BY AUTOPSY*

R. H. Rigdon and Helen Kirchoff

It is difficult to obtain satisfactory data relative to the frequency of cancer. Vital statistics are the primary sources for such in this country; however, the accuracy of these may be open to question. Macdonald (1938) made a study of the accuracy of the cancer death records in Massachusetts and found that "the presence or absence of cancer, disregarding location, indicates that the presence of cancer as measured by the death certificate is accurate to somewhere between 89 and 98 per cent. . . . An estimate that cancer as a disease was accurately diagnosed in 95 per cent of the total cases is felt to be a sound one." The accuracy of diagnosis in the cases reported by Pohlen and Emerson (1942) is not quite so high. They reviewed 4,051 cases from 10 general hospitals in which, either by autopsy or by clinical statement of cause of death, or in the admission diagnosis to the hospital, some form of malignant or unspecified tumor was mentioned. In this series of cases there were 3,462 cases of cancer as demonstrated by autopsy and 271 additional cases in which a malignant tumor was an incidental finding at autopsy. The correct diagnoses varied between 96 and 26 per cent. A higher per cent of accuracy was noted in those cases that could be easily recognized, such as the breast, cervix and rectum, while it was lowest in cases of liver tumor.

Swartout and Webster (1940) expressed the opinion that few studies had been made to judge the possible effect of erroneous clinical diagnoses upon mortality statistics though the value of such data can hardly be questioned. The antemortem diagnosis was correct in 79 per cent of 8,080 cases studied at autopsy in Los Angeles County. These investigators comment on the fact that this percentage "does not seem good

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enough to warrant the use of ordinary mortality data as the basis for some of the fine-spun calculations and predictions that we have seen based on such data." Because of the deficiencies in clinical diagnoses as revealed by comparisons with autopsy findings, all autopsy data in New York City are now reported to the Department of Health (Jacobson, 1948).

The observed variations in the cause of death as reported on the death certificate and that revealed by the autopsy have emphasized to us the possible erroneous data on the frequency in which cancer occurs in this country. Two hundred cases, examined by autopsy between January 4, 1948, and September 27, 1950, in which a malignant tumor was present, were reviewed and the diagnosis made at autopsy was compared with that recorded on the death certificate.

Table I gives the anatomic site, the number and the percentage of each neoplasm as observed at autopsy, and the number of neoplasms reported on the death certificate. It is of interest to note the frequency of the occurrence of neoplasms in the different anatomic sites. The order of frequency in

TABLE I

Anatomic Location of Neoplasms as Studied in 200 Autopsy Cases

Sites	No. Cases Found at Autopsy*		No. Cases Diagnosed on Death Certificates	Percentage Agreement
Brain	27	13.1%	20	74.1
Lung	25	12.1	19	76.0
Leukemia	21	10.2	19	90.5
Prostate	16	7.8	4	25.0
Stomach	14	6.8	9	64.3
Pancreas	11	5.3	8	72.7
Hodgkin's disease	9	4.4	8	88.9
Cervix	8	3.9	5	62.5
Generalized (sarcomata)	8	3.9	3	37.5
Esophagus	7	3.4	6	85.7
Bladder	6	2.9	4	66.7
Liver	6	2.9	3	50.0
Adrenal	6	2.9	2	33.3
Colon and rectum	5	2.4	4	80.0
Kidney	5	2.4	2	40.0
Breast	4	1.9	3	75.0
Skin	4	1.9	1	25.0
Uterus	3	1.5	0	00.0
Miscellaneous sites	21	10.2	12	57.1

*Six cases had two neoplasms.

this study is different to that recently reported by Saxton et al. (1950). These investigators reviewed 2,322 cases of cancer studied at autopsy between 1935 and 1950 in St. Louis and found that 14.1 per cent occurred in the large intestine, 9.1 per cent in the stomach, 10.4 per cent in the lung, 6.4 per cent in the prostate, 4.5 per cent in the esophagus, 4.3 per cent in the pancreas and 4.1 per cent in the brain. As shown in Table I, 6.8 per cent of our cases occurred in the stomach, 12.1 per cent in the lung, 7.8 per cent in the prostate, 3.4 per cent in the esophagus, 5.3 per cent in the pancreas and 13.1 per cent in the brain. The frequency of occurrence of neoplasms in any autopsy series is likely to vary widely since it reflects many factors relative to the type of hospital service. Many of the cases admitted to our hospitals are diagnostic problems from many counties throughout the State of Texas.

Table II summarizes the data obtained in this study. In this table it may be seen that in 132 cases, or 66 per cent, the diagnosis was the same on the death certificate as that recorded from the pathologic study. This percentage does not correctly

TABLE II

Summary of Data on Cases of Cancer Studied at Autopsy

200	Cases with neoplasms as demonstrated at autopsy reviewed.
6	Cases had two neoplasms:
1	Meningioma and carcinoma of the stomach.
1	Meningioma and carcinoma of the uterus.
1	Carcinoma of pancreas and carcinoma of the rectum.
1	Carcinoma of skin and carcinoma of prostate.
1	Malignant tumor of thymus and carcinoma of prostate.
1	Astrocytoma and carcinoma of prostate.
4	Cases—Malignant only by position:
2	Meningiomata.
1	Acoustic nerve tumor.
1	Adenoma of pituitary.
132 (66%)	Cases—Diagnoses same at autopsy and on death certificate.
34 (17%)	Cases—Death certificate did not record a malignant process.
6 (3%)	Cases—Death certificate did not record the primary site of origin correctly.
33 (16.5%)	Cases—A tumor was considered as a primary lesion. However, it was either not recorded or the site was given incorrectly on the death certificate.
17 (8.5%)	Cases—A tumor was considered as an incidental lesion but either was not recorded on the death certificate or the site of origin was incorrectly given. (Eleven occurred in prostate and 1 each in rectum, uterus, cervix, thymus, stomach and brain).

reflect the clinical diagnoses since many of the death certificates did not show a neoplasm that was known to have been present as indicated by the following observations.

Brain: Although the death certificate carried the diagnosis only of "edema of the brain," one of the cases was operated upon a few days before death for a brain tumor. Another case was operated upon and a tumor was demonstrated but the death certificate carried only the diagnosis of "hydrocephalus." There were two cases in which the pathologists could not agree as to the diagnosis of the brain lesion. One occurred in a white male 23 years of age who also had a granulomatous lesion in the lungs. Some considered the cerebral lesion to be neoplastic, while others thought it to be granulomatous. The death certificate recorded this case as a "glioma of brain." The second case was a colored female 65 years of age who had multiple lesions in the brain. Some pathologists considered the case as one of perithelioma of the brain, while others thought it to be a granulomatous lesion. The death certificate carried the diagnosis of metastatic tumor of brain stem and cerebral cortex.

Lungs: There were two cases with advanced pulmonary tuberculosis and primary cancer of the lung. The death certificate in both of these cases gave only tuberculosis. The death certificate in another case showed "brain tumor," although the lesion was a metastatic neoplasm from a bronchogenic carcinoma. The death certificate on another case was "thrombophlebitis with pulmonary embolus," while the autopsy revealed a bronchogenic carcinoma.

Stomach: A patient had a gastrectomy performed for cancer one year preceding the time of his death; however, the death certificate was reported as carcinoma of the esophagus with generalized peritoneal carcinomatoses and bronchopneumonia. In a second case there was a carcinoma of the stomach and the cause of death on the death certificate was given as "coronary infarction with cardiac failure, pulmonary edema and hypostatic pneumonia." It is true that the patient did have a myocardial infarction at death, as well as tumor metastases in the liver. In one of the cases of carcinoma of the stomach the lesion, in our opinion, should be considered only an incidental finding.

Prostate: There are five cases of primary carcinoma of the prostate and eleven in which the neoplasms may be considered to be only an incidental finding. There were two cases diagnosed on the death certificate as carcinoma of the prostate that were not confirmed at autopsy. It is not surprising to find such a large number of nonclinical carcinomata in the prostate since the age of many of our cases is high. Moore (1943) has called attention to the high frequency of such neoplasms in elderly individuals.

Liver: One of the neoplasms in the liver was a sarcoma and it has been reported in the *American Journal of Diseases of Children* (Willeford and Stembridge, 1950). In one case of cirrhosis the carcinoma was only an incidental finding.

Sarcoma: The pathologist frequently encountered some difficulty in arriving at a satisfactory diagnosis in some of these cases of sarcomata. In one case there was a retroperitoneal tumor considered to be a fibrosarcoma by some and a rhabdomyosarcoma by others. One case was diagnosed on the death certificate as a retroperitoneal sarcoma with renal obstruction, while at autopsy it was shown to be a myosarcoma of the uterus.

Kidney: There were only five cases of primary carcinomata of the kidney so diagnosed at autopsy. A nephrectomy had been performed in one case a year before death by an osteopath. Since there was no primary site of origin found in this case at autopsy, the tumor was thought most likely to have originated in the surgically removed kidney. The death certificate on this case stated "metastatic carcinoma and duodenal fistula." A papillary cystadenocarcinoma of the kidney was removed and so diagnosed by the pathologists. Seventeen days later the patient died. Death was attributed to coronary thrombosis and arteriosclerosis without any reference to the renal neoplasm. The third case also had a retroperitoneal sarcoma involving the kidney removed two months before death; however, the death certificate stated "C.V. accident, hypertension and coronary disease."

Skin: The diagnosis made on the death certificate of squamous cell carcinoma of the skin illustrates some of the variations observed in the diagnoses given on the death certificate

and those made by the pathologists. One case had a squamous cell carcinoma involving the cheek. The death certificate recorded it as a carcinoma of the mandible. Obviously, a carcinoma does not arise from bone. A second patient had been treated for a squamous cell carcinoma of the left leg. The death certificate carried only the diagnosis of metastatic squamous cell carcinoma to right and left inguinal regions. One case was diagnosed by the pathologist as carcinoma of the appendages of the skin in the inguinal region and incidentally an adenocarcinoma of the prostate was present. The death certificate on this case carried the diagnosis of uremia, renal failure and nephrosclerosis.

Leukemia: In this study the highest correlation between the diagnosis recorded on the death certificate and that made by the pathologists, where a large number of cases was involved, was in leukemia. Only two cases were diagnosed as leukemia pathologically that did not carry a similar diagnosis on the death certificate. One of these was diagnosed pathologically as lymphosarcoma and the other as reticulum cell sarcoma. Since the term leukosarcoma has been used very frequently in this institution, no attempt was made to separate any case diagnosed as leukemia at autopsy from those diagnosed as leukosarcoma on the death certificate.

Esophagus: The pathologic and clinical diagnoses were similar in seven of the eight cases with carcinoma of the esophagus. The eight cases had been operated upon for carcinoma of the esophagus; however, the death certificate stated only "post-esophagectomy death."

Adrenal: Of the six cases diagnosed at autopsy as a primary neoplasm of the adrenal, only two death certificates carried a similar diagnosis. One of the remaining four was diagnosed as carcinoma of the pancreas, another as lymphatic leukemia and two cases as primary renal tumors.

Cervix: Since the cervix is so readily observed, it is always of interest to know why the death certificate does not carry the diagnosis of primary cancer when such a neoplasm is demonstrated at autopsy. In one case the neoplasm was located in the uterus and not in the cervix. One case was being treated on the Orthopedic Service for a fracture of the femur when

death occurred. The third case of carcinoma of the cervix was diagnosed on the death certificate as "carcinoma of the uterus? cervix? vagina?"

Uterus: There were three cases in which the uterus was considered by the pathologist to be the primary site of the neoplasm without the death certificate giving a similar diagnosis. One of these was considered to be a chorioepithelioma. A hysterectomy had been performed eight years previously and at death there was extensive infiltration of the cervix and vagina. This case has been reported in the *American Journal of Surgery* (Willeford and Rigdon, 1950). An adenocarcinoma of the uterus was an incidental finding in one case. The third case had been diagnosed by biopsy as a myosarcoma; however, the death certificate carried the diagnosis of "retroperitoneal sarcoma with renal obstruction."

Reticulo-Endothelial System: As is frequently the case with neoplasms arising from the reticulo-endothelial system, there was encountered some difficulty in making a pathologic diagnosis in some cases. One was diagnosed by the pathologist as a reticulo-endothelial neoplasm, while the death certificate recorded it only as bronchopneumonia. A second case was diagnosed pathologically as reticulo-endotheliosis, while the death certificate recorded it as a lymphoma or leukemia.

In our study of 200 autopsy cases, we found six (3%) with two neoplasms. Saxton et al. (1950) in 2,322 cases found 85 (3.7%) with two or more neoplasms. Warren and Gates (1932), in 1,078 cancer autopsies, found 40 (3.7%) instances with two or more neoplasms.

This study of correlating the diagnoses as given on the death certificate with that made at autopsy emphasizes the necessity of recording accurately on the death certificate known facts relative to the case. Because such is frequently not done, our vital statistics are of little value in determining the frequency of the occurrence of specific neoplasms. Every practicing physician knows how difficult it is to complete a death certificate when there have been inadequate studies made preceding the time of death. However, what is recorded on the certificate helps to establish the frequency of that disease in this country and the data are used to compare with the mortality

data from other countries throughout the world. Dorn and Horn (1941) said, "The principal effort now should be directed toward improving the information entered upon the certificate, the most important item of which is the cause of death."

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PHYSICAL THERAPY IN FRACTURES

W. A. SELLE*

The treatment of fractures is more than a simple problem of adequate reduction as shown by roentgenograms. It can be and often is a highly complex problem in which treatment of all component parts of the involved extremity and the patient as a whole must be considered as well as the broken bone itself. The fractured ends must be held immobile in constant opposition for union while at the same time motion must be made possible in muscles and joints to aid removal of hemorrhage and exudates, and to prevent muscle atrophy, swelling of joints, stiffness and circulatory changes. Physical medicine is being increasingly used in the solution of these problems.

Various writers have outlined the procedures customarily employed in the treatment of fractures by physical medicine. Knapp indicates that effective physical treatment is adapted to the pathological conditions it is desired to overcome, and points out that the injury accompanying a fracture is not limited to the bony structures, for the force which causes a fracture is transmitted through the bone to the surrounding soft tissues. Trauma to the soft tissues, therefore, is the inevitable accompaniment of fracture of the bone. Hemorrhage, extravasation of lymph or tissue fluid result in swelling which devolps to its fullest extent within a few hours, complicating the fracture and creating a condition which must be treated in addition to the bone. For, if allowed to progress to completion, the organization of blood and tissue fluid culminates in the formation of firm fibrous adhesions between solid structures such as tendon, bone ligament, facia, joint capsule, or joint cartilage. Marked interference with function of the extremity by limitation of joint motion may thus result. According to Murray, such organization is well started within 72 hours and has progressed considerably by 10 to 12 days. Knapp points out that the two most important factors limiting joint motion, other than malunion of bones, are swelling

*From the Department of Medical Physics, Medical School of the University of California at Los Angeles. Received for publication April 20, 1951.

and immobilization. Treatment therefore must begin immediately after the fracture has occurred and not after solidification has set in.

The object of specific physical treatment then is to encourage removal of a major portion of the exudate, to minimize swelling and pain during the period of immobilization, and to prevent fibrous tissue and muscle atrophy. The physical therapeutic measures employed specifically for fractures are chiefly: heat, massage, elevation and exercise. In children, fractures usually heal rapidly and thoroughly so that indications for physical therapy are not so great as in adults.

In addition to local changes resulting from fractures, particularly those of the leg, trauma has deleterious effects on the patient as a whole. These may be physiological and psychological. The physiological effects in general are those caused by inactivity as a result of enforced bed rest during repair of the fracture. Muscles may become atrophied and weak due to disuse. Foot drop in the unaffected leg occurs frequently in older people. Joints may become stiff and finer co-ordinating movements lost. Mineral metabolism is disturbed by inactivity. Decubitus ulcers may appear, particularly in patients who require traction in a fixed position. In patients bed-ridden for long periods, the circulatory system adjusts to a reclining position; considerable time and effort are necessary to re-orient the patient to the erect position. Physical measures must therefore be directed to the patient as a whole as well as to the localized injury.

The pschological effects of prolonged immobilization are also important. The patient develops an attitude of inertia and frequently does little or nothing for himself. Mental depression may occur. Recovery is delayed and some patients develop disabilities out of proportion to their injuries. Frequently the most important function of physical medicine is to convince the patient that he can and must help himself. This may be brought about by first demonstrating to him that movement can be performed and finally convincing him that he can use motion in his daily activities. In the eyes of many, the psychological stimulus is a most important factor in the physical treatment.

Specific Treatment

Physical treatment to any individual fracture case depends on the pathological state. Some authorities divide the treatment methods into those used during the early stages of the fracture and those used later. The first is the stage of organization—lasting usually 2 or 3 weeks—during which appropriate treatment may result in removal of the disturbing elements. The second is the stage of scar tissue and fibrous adhesions which limit function. While the same physical agents—chiefly heat, massage and motion—are used in both stages, the methods employed and the physiological effects expected vary.

Early Stages

1) *Heat*

Heat, like massage and exercise, assists in restoring circulation, on which repair depends. Coulter contends that in the open splinted fractures, taking for granted that the fracture has been reduced and that sufficient time has elapsed for cessation of hemorrhage, it is advisable soon after reduction to apply heat two or three times daily for periods of 20 to 60 minutes. While heat may be applied by a great variety of methods, the easist and most convenient method is by lamps, bakers, or hot compresses. Coulter advocates radiant heat from an electric lamp baker consisting of four 60-w lamps. Such superficial heating is better than the deeper heating by short wave diathermy at this stage, because the latter produces too great an arterial flow and tends to interfere with the lymphatic and venous drainage in the deeper structures, thereby increasing further local circulatory embarrassment and retarding the removal of tissue exudates. Even superficial heating should not be used alone but should always be accompanied or followed by massage, elevation or active muscle contraction. Heat also diminishes pain and muscle spasm; it does so, according to some writers, by reflex action.

2) *Massage*

Lucas-Championniere first pointed out the advantages to be derived from the early use of massage and mobilization in the treatment of fractures. Pemberton and Scull state that: 1) light stroking is valuable in promoting absorption and removal of detritus; 2) deeper treatment promotes relief of lymphedema

and augments flow of blood in areas concerned; 3) depending largely on the relative risk of moving fractured parts, light stroking may be instituted almost immediately following an injury, proceeding gradually through effleurage to deeper and more vigorous massage in late stages. Coulter adds that massage relaxes muscle spasm and has special value in preparing the part for passive and active exercise.

Bierman contends that massage (which can be applied before as well as directly after the reduction of a fracture) causes diminution in pain and discomfort as well as improvement in circulation and acceleration of functional recovery. Repair is thus hastened, joint motion increased, and muscle atrophy diminished. The physiological effects of massage, which vary with the type and dosage employed, are due, according to Mennell, to reflex reaction as well as to mechanical action.

Watson, in advocating the early use of massage and mobilization, states: "Organization occurs during the first nine to ten days, hence it is during this crucial period that the local drainage apparatus, the circulatory and lymphatic systems, already reduced in efficiency by the injury received, should be aided in their work of debris removal. Massage rapidly removes these effusions and extravasations by restoring the local lymphatic and circulatory drainage to its former efficiency."

Bierman stresses that too long an interval is usually allowed to elapse before massage and motion are initiated. He points out that while strenuous and careless application of massage and motion may cause displacement of fractured ends of bone, prolonged immobilization of bone may result in atrophic changes in soft tissue, subsequent limitation of motion and even ankylosis. It would appear logical to start gentle massage early. Coulter points out that the chief purpose of massage at this time is to "relieve reflex disturbance of the vasomotor system, to relax muscle spasm and to prepare for gentle passive or relaxed movement." A bivalved cast permits massage while the part is still immobilized. The first few treatments should be of short duration, the time being increased as healing progresses. Mock has presented a table showing the approximate times at which massage and motion may be instituted in various types of fractures, and cautions that only superficial stroking should be given over the region of the fracture. Evidences of too much massage

are increased tenderness, swelling or stiffness in the adjacent joint.

3) *Exercise*

There is uniform agreement that the most efficient method by which edema and hemorrhage can be removed is active exercise, which obviously is contraindicated when there is danger of displacement of bone fragments. Blair states that alternation of blood supply influences normal calcification of bone and that the alternate ischemia and hyperemia by muscle action may thus favorably affect bone healing as well as remove edema and the products of hemorrhage.

Some orthopedists prefer delayed activity until the bony callus has formed for fear that motion will interfere with union. Knapp points out that the fracture line can usually be immobilized by the surgeon to permit active motion of some portion of the extremity throughout the period of healing, and that even in the more serious cases skeletal traction and internal fixation allow adequate immobilization of the fracture line with minimum immobilization of surrounding joints. This, however, usually requires considerable ingenuity on the part of the surgeon.

Bierman likewise states that the development of improved methods of fixation permits early application of physical measures. These relatively recent techniques include fixation at bone operation, direct bone traction by means of Kirschner wires, pins and tongs, and traction suspension by means of Thomas splint. Plaster-of-Paris casts can be bivalved to allow physical therapeutic procedures to be carried out. There is general agreement that joints whose movements do not interfere with the alignment of the bone ends should not be immobilized. In fractures of the radius and ulna, finger movement should be permitted; the same is true of the toes in fractures of the leg. The time at which mobilization of fractures extremities can be begun obviously varies with the location of the fracture.

Lindsay and Brown state that early mobilization is indicated in those cases in which there is no gross deformity or in which gross deformity is not likely to result from the forces employed in obtaining motion. Incomplete and subperiosteal fractures of the shaft and complete and incomplete fracture in and about the joints are most likely to fall into this classification. In ad-

dition, there are cases in which gross deformity or displacement may be corrected and the position maintained with or without internal fixation. Examples of fractures in which early mobilization may follow reduction and internal fixation are those of the patella and the olecranon.

Wilson calls attention to the fact that delayed union or non-union is more likely to occur in the shafts of the long bones than in fractures through cancellous bones, and especially in fractures of the scaphoid, neck of the femur, and in both bones of the forearm at the middle and upper third. He cautions that immobilization of these regions should therefore be as complete as possible, even though it be at the expense of functional improvement.

Heat and massage are preparatory to exercise which is recognized generally as the most important procedure in physical therapy of fractures. In fact, many writers agree that the great majority of fractures require only active motion for their specific physical treatment.

Exercise may be passive or active. Passive exercise is further subdivided into relaxed motion and forced motion. Active exercise consists of assisted, free and resisted types. At first, exercise should be passive, with the patient's muscles relaxed. The development of pain and muscle spasm are indicative of too vigorous an application of exercise. According to Knapp, relaxed passive motion is hardly ever attainable in early fractures since complete relaxation of muscles is rarely obtained. Because of fear, the patient vountarily or involuntarily contracts his muscle in anticipation of movement, thereby converting passive motion into resisted motion and threatening displacement of fragments. As callus formation becomes more definite, assistive exercises replace the passive.

Knapp states that active exercise is practical usually during the third and fourth weeks following fracture; he adds that if adequate immobilization can be obtained by tight-fitting plastic casts or internal fixation, free active or even resisted active motion may be used, especially in the performance of ordinary daily tasks that do not require great strength or heavy lifting. Knapp points out the difficulty of convincing the patient that he can carry out active motion without harming himself. The difficulty of obtaining this mental attitude he attributes to the lay

opinion that fractures must be kept quiet. The various movements which may be employed are adequately described in most textbooks of physical medicine. Voluntary exercise derived from occupational therapy is usually more interesting and therefore more valuable than set forms of routine exercise.

Movement of joints in close proximity to the fracture line should be carried out by assisted active motion. Such exercise obviously must be conducted with great care and implies full co-operation between the orthopedic and physical medicine sections. The technician supports the part to eliminate gravity and the patient carries out the motion within the limits of pain. Under these conditions, according to Knapp, the patient stops the motion at the earliest sign of pain and there is little danger of disturbing the fragments; only that range of motion attained without pain is allowable.

If swelling is not removed satisfactorily by active motion, or if active motion is impossible, the next most effective method, according to Knapp, is the use of elevation to allow gravity to assist the venous return. Also, where voluntary motion is impractical or impossible, electric stimulation may be used to prevent muscle atrophy and circulatory disfunction. Faradic current is employed if the nerve is intact, galvanic or sinusoidal if the nerve is severed.

If muscle atrophy and fibrosis is to be prevented and limitation of motion is kept minimal, active motion of all joints not necessarily immobilized must be insisted upon between treatments throughout the course of fixation. Talbot states that early gentle manipulation of the neighboring joints prevents stiffness which follows splinting; that in supraspinatus tears at the shoulder, unless passive movements are commenced in 10 days, great limitation results; that fractures of the upper humeral shaft require immobilization for three weeks before minor manipulations are begun; and that supracondylar fractures of the humerus must be left at least 10 days in the reduced state before daily progressive manipulation is adopted.

Since physical therapy is recommended during the first two weeks in order to obtain the removal of hemorrhage and exudates, and since this is the period of greatest danger of displacing bone fragments, Wilson emphasizes that the treatment be applied by the surgeon or under his direct and careful supervision.

Later Treatment

In the later stage, after the bony callus has become firm and fibrous formation initiated, a more vigorous and definite treatment becomes necessary in order to prevent adhesions and atrophic changes in muscles and joints.

1) *Heat*

This is used for softening fibrous adhesions, as well as for the physiological effects mentioned previously. Short wave diathermy is not contraindicated at this stage and may be extremely helpful, although many physiatrists recommend moist heat. Whirlpool bath is particularly valuable, especially as a preparation for subsequent massage and manipulation in painful, stiff joints. Motion which could not otherwise be tolerated may be given in the bath. Hot packs are useful about the shoulders. Infra-red seems to be less valuable during late treatment than early.

2) *Massage*

Massage (which follows heat) now includes deep stroking and kneading which counteracts stasis. In general the treatment is more vigorous than that previously mentioned but is not viogorous to the point of creating muscle spasm. Massage at this time aids in stretching adhesions so that greater range of motion is allowed and nutrition is improved. Massage tends to reduce swelling and tenderness. When active exercise is permissible, massage can be reduced ort eliminated as muscle contraction is more valuable in obtaining return of function.

3) *Exercise*

Assistive active motion, free motion and resisted active exercise are used progressively as the patient improves. Again, the voluntary exercise derived from interesting occupational therapy is often more valuable than set forms of routine exercise, although the latter should be encouraged. According to De Lorme, "weakened atrophied muscles should not be subjected to endurance building exercises until muscle has been restored to normal by power building exercises." De Lorme maintains that high-resistance, low-repetition exercises produce power while low-resistance, high-repetition exercises produce endurance.

General Treatment

As pointed out, it is now recognized that recovery of the injured depends not only on recovery of the local injury but also on maintenance of activity throughout the patient's body. This type of physical medicine or rehabilitation is started as early as practical and as soon after the fracture has been reduced as possible. It consists primarily of exercises designed to maintain activity, muscle tonus and muscle power in those parts of the body not involved in the immediate injury. Such conditioning or anti-deconditioning exercises are well known but are not as widely employed as might be expected. Exercises of this type can be effectively conducted in groups since general exercise *en masse* permits competition which makes for more enjoyable activity. Such rehabilitation exercises, now widely used in Veterans Administration hospitals, are important not only for the physical effects produced but also for the psychological effects; they give incentive for activity and tend to keep the patient aware of the fact that he is not totally disabled, that he can make useful motions and that he will be able to carry on daily vocational activities at an early time. These exercises progress logically to more specific types of occupational therapy, which essentially consists of activity of a voluntary, purposeful kind.

Occupational Therapy

Occupational therapy is the link between physical therapy and return to ordinary working conditions. Ordinary daily physical therapy lasts 60 to 90 minutes at the most; specific mechanical exercises therefore have definite limitations. In many cases, the patient to be treated not only is physically handicapped, in that muscular control has not been regained, but is frequently uninterested in using the stiff joints and atrophied muscles. If any interest can be aroused by use of hands, arms or legs in a handicraft or muscular skill, the patient benefits through a process of re-education or reactivation for the whole muscular and neural system, psychologic as well as physiologic. It is on this basis that occupation and play are introduced.

Occupational therapy is divided into recreational and vocational activities. Adaptation of occupational devises to treat-

ment of the involved portions are employed, enabling the patient to use desired muscles and joints, and resulting in strengthened muscles and increased range of motion. The part involved in occupational therapy is prepared for activity by preliminary treatment with heat, massage or exercise. Movements are gradually increased in extent and complexity until the patient is able to participate in full employment. It is not difficult to convince a man that he is able to leave the hospital when he knows that he is capable of carrying on activities equivalent to those of his old job.

Summary

Physical treatment of fractures is directed to the soft tissue injury and to the total patient as well as to the bony lesion itself. More specifically it is directed toward: 1) Prevention or reduction of persistent swelling during this period of immobilization and the resultant limitation of motion; 2) Reduction of pain and muscle spasm; 3) Improvement of circulation and nutrition; 4) Prevention of mental and physical impairment, and 5) Early return to useful occupation.

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POTASSIUM, SODIUM, WATER CONTENT AND ADENOSINETRIPHOSPHATASE ACTIVITY OF VAS DEFERENS FROM NORMAL AND CASTRATED RATS*

J. R. Valle,† J. Leal Prado and Paul L. Ewing

Castration in rats is accompanied by increased excitability and characteristic pharmacological reactivity of the smooth muscle of male genitals. Whereas the vas deferens from castrated rats exhibits automatism and tonic rhythmic response to acetylcholine, pilocarpine and muscarine added to the nutritive bath, the vas deferens from normal donors is quiescent and unreactive to those drugs of the parasympathomimetic group (Martins and Valle, 1939; Martins, Valle and Porto, 1939). The rôle of potassium in muscular contraction (Fenn, 1940), its liberation by the smooth muscle of rats after acetylcholine (Cicardo, 1941) and previous work searching to correlate those changes with the acetylcholine content of male rat's genitals (Valle and Porto, 1943), led to the comparative analysis of the potassium content of vasa deferentia from normal and castrated rats. Since the eventual liberation of potassium after castration could be accompanied by sodium and water retention, these two constituents were also estimated. On the other hand, concentration of adenosinetriphosphatase (ATPase) being an important factor for the muscular processes (Engelhardt, 1946), deferens homogenates were also studied concerning their ATPase activity.

Material and Methods

Fifty-one male, adult rats of the 2BAW strain of this laboratory, weighing from 207 to 440 g., 12 normals, 16 hemicastrates and 23 castrates were used. Castration was performed under ether anesthesia through suprapubic approach. Maintenance

*From the "Laboratorios de Farmacologia e Bioquímica, Escola Paulista de Medicina," São Paulo, Brasil, and the Department of Pharmacology, The University of Texas Medical School, Galveston, Texas. Received for publication March 18, 1951.

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of the vas deferens with its normal connections was achieved by careful removal of the testis without the epididymis.

Ten to thirteen days after the operation the rats were killed by decapitation and the vas deferens carefully removed, stripped from its connective tissue and rid of its secretion. The organs were pooled according to the hormonal conditions of the donors, each collection weighed on a torsion balance and separated in two groups. One was used for the estimation of potassium, sodium and water content and the other one minced in a glass homogenizer before assaying the ATPase activity. The first group was previously dried in an oven at 110° C. for 12 hours and the decrease in weight taken as the water content. Potassium was determined according to the method of Marenzi and Gerschman (1938), sodium by the method of Marenzi, Gerschman and Villallonga (1942). Previous digestion was performed by following Pereira's procedure (1945). Results are given in mg. per cent of wet tissue.

Adenosinetriphosphatase activity of the vas deferens homogenates was assayed according to the method employed by Singher and Millman (1947). Figures refer to micrograms of phosphorus liberated by 1 mg. of fresh tissue.

Results and Comments

Pertinent data appear in Table 1. Castration seems not to interfere with the potassium, sodium and water content of the vas deferens. Furthermore, adenosinetriphosphatase activity of deferens homogenates remains practically the same as before gonadectomy. Therefore, lack of circulating androgens, at least within the first 13 days after bilateral castration, does not alter the concentration of those constituents of the smooth muscle of male rat's genitals.

Despite the fact already mentioned that castration induces in rats a characteristic behavior "in vitro" of the male genitals, viz., higher excitability and reactivity to drugs of the vagomimetic group, these changes are unaccompanied by significant increase or decrease of the potassium, sodium and water content of the vas deferens. Miller (1943) had shown that testosterone propionate under certain conditions may change the potassium content of rat's muscle. Increased sodium retention and increased potassium excretion would depend on the duration of

treatment, these changes being more pronounced after desoxycorticosterone. According to Fleckenstein (1948) muscular contraction would follow Na substitution by K on the surface of the muscle fiber. The type of experiment here reported does not warrant the assumption that this would occur on the surface of the vas deferens fiber following castration. Even if significant shift was registered in K, Na and water content of the male rat's genitals after orchiectomy, it would be difficult to correlate such a finding with the pharmacological changes described before.

TABLE I

Constituents of Vas Deferens from Normal, Hemicastrated and Castrated Rats

Groups	Number of Rats	K mg %	Na mg %	Water %	ATP-ase mg P/mg*
		A—Normals (12)			
10–A1	3	402	105	72	10.1
16–B1	3	513	195	78	11.1
34–C1	3	450	129	80	...
40–D1	3	470	...	79	10.8
		459 ± 46§	143 ± 38	77 ± 4	10.7 ± 0.5
		B—Hemicastrates (16)			
9–A2	3	440	121	76	10.5
15–B2	3	452	195	77	10.5
32–C2	3	465	140	77	...
32–E1	3	440	112	72	...
40–F1	4	425	...	76	9.6
		444 ± 15	142 ± 37	76 ± 2	10.2 ± 0.5
		C—Castrates (23)			
10–A3	3	492	152	74	10.1
15–B3	3	450	152	69	11.6
33–C3	4	414	121	72	...
33–D2	5	433	123	74	...
39–E2	4	444	...	72	9.5
39–F2	4	380	...	75	11.3
		435 ± 38	137 ± 18	73 ± 5	10.6 ± 0.9

*Micrograms of Phosphorus liberated by 1 mg of fresh tissue.
§Standard deviation.

Csapo (1950) had shown that following ovariectomy in rabbits the contractile actomyosin system of the uterus and the adenosinetriphosphatase activity decrease, the administration of estrogen promoting a recovery of both. Singher and Millman (1947) have found, on the other hand, that ATP-ase activity of uterine muscle of rats seems to be inhibited by testosterone. Despite these facts and under our experimental conditions no changes were recorded in the ATP-ase activity of deferens homogenates after castration.

Summary

Increased excitability and characteristic pharmacological reactivity "in vitro" of the smooth muscle of male rats following castration are unaccompanied by changes in potassium, sodium and water content and ATP-ase activity of vas deferens homogenates. The mean values for each constituent in both hemicastrated and normal control groups were not significantly different from those given by the castrated donors.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

June, 1951

Basic: A Babics discusses lymphatic circulation of kidneys (*Acta Med Acad Sci Hungarica* 2/1/51). AD Bass & Co show sulfa drugs, lithium salts & nitrogen mustards inhibit fetal development by interfering with sex hormones (*J Pharmacol* 101/362/51). P Buffa & Co describe biochemistry of fluoroacetate poisoning (*Biochem J* 48/467/51). F Coste criticizes (*Presse Med* 59/481/April 1/51) H Selye's theory of adaptation to stress (*Brit Med J* 1/1383/June 17/50). SC Cullen & EG Gross confirm anesthetic action of xenon (*Science* 113/580/May 18/51). JH Dibble analyses liver degeneration, necrosis & fibrosis (*Brit Med J* 1/833/Apr 21/51). Our JC Finerty & TC Panos show genetic factor in parabiosis intoxication (*Proc Soc Exp Biol Med* 76/833/51). ZZ Godlowski finds heparin blood clot inhibition prevents ACTH or adrenocortico steriod eosinopenia (*Brit Med J* 1/854/Apr 21/51). HN Green & HB Stoner summarize *Biological Actions of the Adenine Nucleotides* (HK Lewis, London, 51, 221pp, 25s). F Hoffman & Co show quantitative relation between nitrogen excretion & oxygen consumption (*Acta Physiol Latino-Amer* 1/84/51). C Huggins & Co discuss gel effects of urea on sulfhydry (*Nature* 167/592/Apr 14/51). RA Huggins & Co find vasodilation with various morphine derivatives (*Arch Internat Pharmaco* 86/112/51). RD Keynes notes potassium leaves active nerve (*J Physiol* 113/99/51). F Lippay & Co discuss spleen in adrenaline theory of vasomotor reversal (*Austral J Exp Biol Med Sci* 28/517/50). K Meyer & MM Rapport analyse mucopolysaccharides of mesenchyme ground substance (*Science* 113/596/May 25/51). RD Passey & Co note high incidence of particles in milk & tissues of human breast cancer by electron microscopy (*Nature* 167/643/Apr 21/51). CM Plum shows metallic injury to liver is reflected by macrocytic anemia (*Arch Internat Pharmaco* 86/52/51). D Richter & RP Hullin find enzyme content of human cerebral cell nuclei & cytoplasm similar with alk phosphatase high in nuclei (*Biochem J* 48/406/51). MF Sartori reviews new war gases including nitrogen mustards, fluoroacetates & phosphates (*Chem Rev* 48/225/51). RS Siffert finds alk phosphatase involved in calcifiable bone matrix but unrelated to loss of chondroitin (*J Exp Med* 93/415/51). RT Simmons & R Jakobowicz analyse Lewis blood antigens & antibodies (*Med J Austral* 1/497/Apr 7/51). GH Smith & JC Opsahl review adrenal hyaluronidase relation in infection (*Yale J Biol Med* 23/361/51). Our HG Swann & Co describe effect of renal venocclusion on intrarenal pressure (*Proc Soc Exp Biol Med* 76/773/51). A Winterstein & Co note heparin is inactivated by depolymerization with fragments appearing in urine (*Helv Physiol Pharmacol Acta* 9/24/51).

Clinic: H. Binswanger applies Selye's stress syndrome to psychiatry (*Schw Med Wchnschr* 81/413/May 5/51). Note *Ciba Symposium on Toxemias of Pregnancy Human & Veterinary* (JA Churchill, London, 51,

455pp, 21s). JB Cleland suggests urinary clinical lab tests for vitamin deficiencies (*Med J Austral* 1/468/Mch 31/51). F Cole proposes intravenous oxygen in severe shock (*Anesth* 12/181/51). JB Foote & NF Maclagan suggest thick-neck clearance test for thyroid function *Lancet* 1/868/Apr 21/51). EJ Gadson reviews glutamic acid & mental deficiency (*Am J Mental Dis* 55/521/51). G Gale discusses management of intestinal obstruction (*Med J Austral* 1/317/Mch 3/51). RS Illingworth wisely advises on sleep problems in young children (*Brit Med J* 1/722/Apr 7/51). WD Keidel analyses auscultation phenomena (*Arch Kreislauf* 17/72/51). E Kerpel-Fronius & Co find anoxia parallels dehydration in infantile toxicoses (*Acta Paed* 40/10/51). WDM Paton discusses glangion-blocking drugs (*Brit Med J* 1/773/Apr 14/51). GC Robinson pleads for personality in managing illness (*Bull Johns Hopkins Hosp* 88/352/51). JF Seybolt & Co describe cytological diagnosis of gastric cancer (*Cancer* 4/286/51). K Singer & Co note abnormal hb in sickle-cell anemia (*Blood* 6/413,429/51.) G Tondury discusses genesis of hare-lip (*Acta Anat* 11/300/51). JW Waldron & Co show cream is local hemostatic & recommend early use in gastroenteric bleeding (*Gastro-enterol* 17/360/51). F Wuhrmann claims relation between nephrotic & myocardosis syndromes (Arzt Forsch 5/153/51).

Etc.: WR Brain appropriately discusses mind & matter (*Lancet* 1/863/Apr 21/51). S Chase finds *Roads to Agreement* (Harper, NY, 51, 250pp. $3.5). AL Kroeber concludes western culture is reconstituting itself (*Proc Am Philosoph Soc* 95/100/51). V Lecuna compiles for Banco de Venezuela *Selected Writings of Bolivar* (Colonial Press, NY, 51, 2 vols, 822pp). R Shaw describes coypright (*Science* 113/571/May 18/51).

July, 1951

Clinical: A Bergstrand indicates disturbance in protein metabolism is common factor in nephrotic syndromes (*Acta Path Microb Scand* 28/139/51). I Bieber discusses pathological boredom & inertia (*Am J Psychother* 5/215/51). HR Bierman & Co successfully achieve human cross circulation noting clinical improvement in leukemia (*Blood* 6/487ü51). DA Campbell & Co observe *Eyes in Industry* (Longmans, London, 51, 420pp, 30s). YT Chang finds crystalline yatanoside fr Brucea sumatrana a potent safe amebacide (*Chinese Med J* 69/87/51). JWL Doust claims anoxia correlates with psychiatric states involving awareness (*Proc Roy Soc Med* 44/347/51). FA Freyhan & Co think senile psychoses follow decreased cerebral blood flow (*J Nerv Ment Dis* 113/449/51). BH Glover discusses behavior patterns & therapy of University homosevuals (*ibid* p 377). EW McChesney & Co recommend ion exchange resins in edema (*Med* 30/183/51). O Nordenfelt describes EKG in cardiac aneurysm (*Acta Med Scand* 139/368/51). SAN Pere shows effective digitalis or novurit therapy in congestive failure causes increased thromboplastin in blood as edema disappears with increasing tendency to thrombo-embolic complications (*Acta Med Scan* 139/Suppl 251/65pp/51). PV Peterson proposes spasmolytic analgesic mixing diphenyldimethyl-amino butene (A29) with hydroxy-phenylmethyl-piperidin-ethyl ketone (A21), in probably addic-

tive "Ketogan" (*Acta Pharmacol Toxicol* 7/51/51). R Platt discusses renal failure (*Lancet* 1/1239/June 9/51). GK Strode edits important *Yellow Fever* (McGraw-Hill, NY, 51, 710pp, $9.5). H Van Canwenberge & C Heusghem find high excretion of reducing steroids in salicylate therapy (*Lancet* 1/771/Apr 7/51).

FUNDAMENTAL: CH Bamford summarizes data on molecular configuration & physical properties of polypeptids & proteins (*Proc Roy Soc Med* 44/393/51). JAV Bates finds no change in cortico-electro activity preceding voluntary movement (*J Physiol* 113/240/51). R Blowers & Co describe erythrocyte flicker correlated with cation transport (*J Physiol* 113/228/51). H Bortels dilates on sun action, physical-chemica lreactions & biological phenomena (*Naturwiss* 38/165/51). SE Brolin analyses steroid action in thymus involution (*Acta Anat* 11/586/51). TO Casperson offers *Cell Growth & Cell Function: A Cytochemical Study* (WM Norton, NY, 51, 185pp, $5). SE Dicker shows antimitotic urethane & clochicine have diuretic action (*Brit J Pharmacol* 6/169/51). D Sckert, M Paasonen & A VVartianien find histamine responsible for skin reaction to mosquito & gnat bites (*Acta Pharmacol Toxicol* 7/16/51). LB Flexner, edits discussion on fluid & electrolyte distribution (*Methods in Medical Research,* Year-Book, Chicago, 51, 306pp, $7). G Gomori edits section on histochemical staining methods *(ibid).* Sir Charles Harrington discusses role of basic science in medical research (*New Eng J Med* 244/777/May 24/51). A Iannaccone & V de Filippis find ACTH increases tubular reabsorption of glucose & renal threshold for glucose (*Fol Endocrin* c/ 919/50). HE Ives shows principle of constancy of velocity of light may be abandoned (*Proc Am Philosoph Soc* 95/125/51). HH Kelley analyses communication (& criticism) in experimentally created hierarchies (*Human Relations* 4/39/51). F Leibetseder & H Kwerch note autonomnc nervous system responses to urethane (*Ztschr Klin Med* 148/180/51). Our CM Pomerat edits section on Tissue Culture Methods (*Methods in Medical Research,* Year-book, Chicago, 51, pp198–291). Note Symposium on Problems of Speech Comprehension (*Nery Child* 9/1–88/51). A Symposium on Public Health Lab Service (Brit Med Bull 7/147–212/51). ML Tainter edits symposium on papain (*Ann NY Acad Sci* 54/143–296/51). P Tolentino shows traumatization of erythrocytes causes hemolysis without fragmentation (*Nature* 167/905/June 2/51). GNM Tyrrell in *Homo Faber* studies human mental evolution (Methuen, London, 51, 288pp, 15s). M Vogt finds plasma potassium increase accelerates adrenal cortical secretion (*J Physiol* 113/129/51). LP Winterbotham discusses primitive medical art & medicine men ofAustralia (*Med J Austral* 1/461/Mch 31/51).

WARM SUMMER READING: RC Andrews gives *Heart of Asia* (Duell, *gation* (Norton, NY, 51, 181pp, $3). V Peterson says "no" to *Gambling:* Sloan, NY, 51, 224pp, $3). WIB Beveridge offers *Art of Scientific Investi-Should It Be Legalized?* (CC Thomas, Springfield, Ill, 51, 158pp, $3). P Valaer notes *Wines of the World* (Abelard Press, NY, 51, 576pp, $6.5).

AUGUST, 1951

10TH ANNIVERSARY NUMBER

The Mid-Decades: One might be pardoned for wondering whether or not an effort such as this is worth-while. After ten years of it a tentative affirmative might be ventured. The War Years: devastation, threats of totalitarianism, authority, power, purges, aggressions—yet the democratic principle remains based on knowledge. Whoever may contribute to the spread of exact knowledge is doing something worth-while. Nuclear fission, medical physics, antibiotics, cellular chemarchitecture, steroids, cerebral function—these fields and so many more well explored during the decade have provided intellectual adventure of the richest kind. To share with those who may be interested is a joy. Such is the recompense.

Clinical: FH Allen & Co describe rapid accurate method for blood grouping of large numbers of people (*New Eng J Med* 244/925/June 21/51). SH Belgorod & Co show thiosemicarbazone "Tibione" (amithiozone NNR) useful in tuberculosis alone or in combination (*Dis Chest* 20/1/51). E Cuboni reviews toxoplasmosis (*Boll Ist Sieroter Milan, Arch Microb Immunc* 30/198/51). FW Ellis & PM West find variations in chymotrypsin & rennin serum inhibitors correlated with clinical state of cancer patients (*J Clin Invest* 30/547/51). L Hirszfeld notes antihistaminics are useful in premature labor & habitual abortion (*Gyn* 131/129/51; *Schw Ztschr Allg Path Bact* 14/133/51). K Inouye & Co think serum cobalt reaction useful in early diagnosis of liver cirrhosis (*Acta Schol Med Kioto* 38/93/51). H Lagerlof & Co analyse orthostatic changes in pulmonary & peripheral circulation (*Scand J Clin Lab Invest* 3/85/51). H Lehman finds gene factors in sickle cell anemia (*Nature* 167/931/51). O Masek recommends pervitin (methyl amphetamine) in acute barbital poisoning (*Lekarske listy* 6/326/51). R Masure finds H_2S is cause of kerato-conjunctivitis in viscose rayon workers (*Rev Belge Path Med Exp* 20/297/51). MF Perutz & Co study hemoglobin in sickle cell anemia (*Nature* 167/929/51). EJ Pulaski & JR Shaeffer review antibiotic therapy in surgical infection (*Internat Abst Surg* 93/1/51, *SGO* suppl July 51). LJ Regan discusses legal authorization for autopsy (*Ann West Med Surg* 5/287/51). MD Siperstein & Co show cholesterol may be synthesized in arteries & contribute to arteriosclerosis (*Science* 113/747/June 29/51). Symposium on chronic beryllium poisoning & treatment with ACTH & cortisone (*Arch Ind Hyg Occup Med* 3/543/51). LE Wexburg includes alcoholics in addiction personality types with low threshold for frustration (*Quart J Stud Alc* 12/217/51).

Preclinical: American Chemical Society announces 75th jubilee meeting NY Sept 3–7: Section on Medicinal Chemistry offers 100 reports from antibiotics to vitamins, with session on chemotherapy by valiant Blakians of San Francisco. AL Baron writes *Handbook of Antibiotics* (Reinhold, NY, '51, 303pp, $6.5). F Bergersen finds cross resistance to antibiotics after exposure to aureomycin (*Nature* 167/996/June 16/51). G Biozzi & Co show low doses of antihistaminics inhibit increase of vascular permeability induced by histamine but not by leukotoxine (*Arch Internat Pharmaco* 86/

335/51). A Burger compiles *Midicinal Chemistry* (Interscience Publ NY 51, 2 vols, 1150pp, $20). JN Davidson & Co review nucleic acid content of cell (*Lancet* 1/1287/June 16/51). RE Davies analyses HCl production by stomach (*Biol Rev* 26/87/51). L Ericksen offers notes on prophyrin pigments (*Scand J Clin Lab Invest* 3/121,128,135/51). KJ Franklin well discusses economy of circulation (*Brit Med J* 1/1343/June 16/51). ES Goldensohn & Co show slow-change brain voltage rise in CO_2 administration & fall in anoxia (*EEG Clin Neurophysiol* 3/231/51). MA Lessler concludes Feulgen reaction (*Ztschr Physiol Chem* 135/203/1924) shows actual site of desoxypentose nucleoprotein in cell (*Arch Biochem Biophys* 32/42/51). I Pataky & KA Pfeiffer find muscle creatine increase following adrenaline & thyroxine (*Acta Physiol Acad Sci Hung* 2/71/51). T Rosenberg analyses splitting of energy rich phosphate bonds (*Rep Steno Mem Hosp* 4/59/50). RH Smithwick & CW Robertson believe hypersensitivity to posture & cold correlate with tendency to hypertension (*Angiol* 2/143/51). BP Wiesmer & J Yudkin find evidence of vitamin B_{12} as part of liver reproductive factor (*Nature* 167/979/June 16/51).

Relaxing Reading: HF Blum's *Time's Arrow and Evolution* is hot (Princeton Press, 51, 222pp, $4). RL Carson revels in *The Sea Around Us* (Oxford Univ Press, NY, 51, 230pp, $3.5). AS Clifton reflects on *Time of Fallen Blossoms* (Knopf, NY, 51, 239pp, $3). L Diat describes *Sauces, French & Famous* (Rinehart, NY, 51, 170pp, $2.5). K Detzer tells about great Louisville Public Library & L Evans extols public libraries as ramparts of freedom (*Sat Rev Lit*, July 7/51). B Engle & TM French reflect psychodynamically on Life & Writings of Solon (638–558BC) *Psychoanal Quart* 20/253/51). C Kligerman dittoes on JJ Rousseau (1712–1778) (*ibid* p 237). P Valaer treats of *Wines of the World* (Abelard Press, NY, 51, 576pp, $6.5).